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RESEARCH INSTITUTE, NEW DELHI.

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**Indian Agricultural Research Institute,**  
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# JOURNAL

OF THE

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PROCEEDINGS OF THE FIFTY-FIRST ANNUAL  
CONVENTION OF THE ASSOCIATION OF  
OFFICIAL AGRICULTURAL  
CHEMISTS, 1935

The fifty-first annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington D.C., November 11, 12, 13, 1935.

The meeting was called to order by the president, F. C. Blanck, U. S. Bureau of Chemistry and Soils, Washington, D.C., on the morning of November 11, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE  
REFEREES OF THE ASSOCIATION OF OFFICIAL  
AGRICULTURAL CHEMISTS FOR THE YEAR  
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*Recommendations of Referees*

(Figures in parentheses refer to year in which appointment expires.)

H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*

SUBCOMMITTEE A: H. R. Kraybill (1936), (Purdue University, Lafayette, Ind.), *Chairman*; G. L. Bidwell (1938) and G. E. Grattan (1940). [Standard solutions; insecticides, fungicides, and caustic poisons (fluorine compounds); soils and liming materials (hydrogen-ion concentration—alkaline soils and acid soils, liming materials, less common metals in soils, fluorine, selenium); feeding stuffs (fluorine, stock feed adulteration, mineral mixed feeds, moisture, hydrocyanic acid in glucoside-bearing materials, biological methods for determination of vitamin D carriers, biological methods for vitamin B complexes, technic and details of biological methods, vitamin D carriers, carotin, qualitative tests for proteins); fertilizers (phosphoric acid, nitrogen, magnesium and manganese, acid and base-forming quality, potash); plants (less common metals, total chlorine, carbohydrates, forms of nitrogen, sodium and potassium); lignin,



enzymes, pepsin, papain, paints, paint materials and varnishes (accelerated testing of paints); vitamins (vitamin A, vitamin D, vitamin D standard); leather and tanning materials, disinfectants.]

SUBCOMMITTEE B: A. E. Paul (1936), (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman*; L. B. Broughton (1938) and H. J. Fisher (1940). [Naval stores (rosin, turpentine); radioactivity, drugs (microchemical methods for alkaloids, microchemical methods for synthetics, hypophosphites, santonin and phenolphthalein tablets, benzyl compounds, guaiacol, rhubarb and rha-ponticum, hexylresorcinol, ergot alkaloids, iodine ointment, biological testing, acetphenetidin in presence of caffeine and aspirin, pyridium, aminophylline, gums, dinitrophenol, cinchophen and sodium bicarbonate tablets, theobromine calcium, chlorbutanol, aspirin and phenolphthalein mixtures, stability of volumetric solutions of potassium iodide, homatropine tablets.]

SUBCOMMITTEE C: W. B. White (1936), (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. O. Clarke (1938) and G. G. Frary (1940). [Dairy products (butter—preparation of sample and fat, cheese, malted milk, dried milk, milk proteins, lactose in milk, gelatin, sediment in cream and butter); oils, fats and waxes (refractometric determination of oil in seeds, hydroxyl number and acetyl value, thiocyanogen value); eggs and egg products (unsaponifiable constituents and fat, detection of decomposition; glycerol, sugar, and added salt, dried eggs); metals in foods (arsenic, copper, zinc, fluorine, lead, mercury, selenium); canned foods, vinegars (ash), meats and meat products, gums in foods, spices, microbiological methods—canned foods (treatment of unopened container, sampling inoculum, culture media of non-acid products and sugar, culture media of acid products, incubation periods and temperatures for cultures); nuts and nut products; fish and other marine products.]

SUBCOMMITTEE D: J. W. Sale (1936), (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. A. LeClerc (1938); and W. C. Jones (1940). Sugars and sugar products (honey, maple products; drying, densimetric, and refractometric methods; polariscopic methods, chemical methods for reducing sugars, lead precipitate); waters, brine, and salt (mineral salts and effervescent salts); alcoholic beverages (beers, wines, cordials and liqueurs; whiskey, rum, and brandy; fusel oil, methyl alcohol; malt beverages, extracts, and sirups, and brewing materials); food preservatives; coloring matters in foods, fruits and fruit products (soluble solids and effect of acids on sugar on drying, electrolytic titration of acidity, fruit acids); flavors and non-alcoholic beverages, cacao products, baking powders and baking chemicals, cereal foods (ash in flour, macaroni products, and baked products; H-ion concentration of flour, starch in flour, flour-bleaching chemicals, CO<sub>2</sub> in self-rising flour; unsaponifiable constituents, baked and macaroni products; milk solids in milk bread, viscosity of flour, cold water extract flour, ergot in flour, catalase and proteolytic enzymes, color in flour); microchemical methods.

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*General referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

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a. ALKALINE SOILS:

*Associate referee:*

b. ACID SOILS:

*Associate referee:* M. F. Morgan, Agricultural Experiment Station,  
New Haven, Conn.

LIMING MATERIALS:

*Associate referee:* W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS (FLUORINE):

*Associate referee:* J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

SELENIUM IN SOILS:

*Associate referee:* K. T. Williams, Bureau of Chemistry and Soils, Washington, D. C.

FEEDING STUFFS:

*General referee:* L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

FLUORINE:

*Associate referee:* Dan Dahle, Food and Drug Adm., Washington, D. C.

STOCK FEED ADULTERATION:

*Associate referee:* P. B. Curtis, Agricultural Experiment Station, Lafayette, Ind.

MINERAL MIXED FEEDS:

*Associate referee:* H. A. Halvorson, Old Capitol Building, St. Paul, Minn.

MOISTURE:

*Associate referee and Chairman:* H. A. Halvorson; G. L. Bidwell and P. B. Curtis.

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS:

*Associate referee:* R. A. Green, University of Arizona, Tucson, Ariz.

BIOLOGICAL METHODS FOR DETERMINATION OF VITAMIN D CARRIERS

*Associate referee:* W. B. Griem, Department of Agriculture and Markets, Madison, Wis.

BIOLOGICAL METHODS FOR VITAMIN B COMPLEXES:

*Associate referee:* C. A. Elvehjem, Dept. of Agricultural Chemistry, Madison, Wis.

TECHNIC AND DETAILS OF BIOLOGICAL METHODS, VITAMIN D CARRIERS:

*Associate referee:* L. L. Latchat, Dept. of Agriculture, Dairy and Food, St. Paul, Minn.

CARROTIN:

*Associate referee:* V. E. Munsey, Food and Drug Adm., Washington, D. C.

QUALITATIVE TESTS FOR PROTEINS:

*Associate referee:* D. Breese Jones, Bureau of Chemistry and Soils, Washington, D. C.

**FAT IN FISH MEAL:**

*Associate referee:* R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

**FERTILIZERS:**

*General referee:* G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

**PHOSPHORIC ACID:**

*Associate referee:* W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

**NITROGEN:**

*Associate referee:* A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

**MAGNESIUM AND MANGANESE:**

*Associate referee:* J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

**POTASH:**

*Associate referee:* H. R. Kraybill, Agricultural Experiment Station, Lafayette, Ind.

**ACID AND BASE-FORMING QUALITY:**

*Associate referee:* L. E. Horat, Agricultural Experiment Station, Lafayette, Ind.

**PLANTS:**

*General referee:* O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

**LESS COMMON METALS:**

*Associate referee:* J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

**TOTAL CHLORINE:**

*Associate referee:* H. L. Wilkins, Bureau of Plant Industry, Washington, D. C.

**CARBOHYDRATES:**

*Associate referee:* J. T. Sullivan, Agricultural Experiment Station, Lafayette, Ind.

**FORMS OF NITROGEN:**

*Associate referee:* H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

**SODIUM AND POTASSIUM:**

*Associate referee:* O. B. Winter.

**LIGNIN:**

*General referee:* M. Phillips, Bureau of Chemistry and Soils, Washington, D. C.

**ENZYMES:**

*General referee:* A. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

**PEPSIN:**

*Associate referee:* A. K. Balls.

PAPAIN:

*Associate referee:* T. L. Swenson, Bureau of Chemistry and Soils, Washington, D. C.

PAINTS, PAINT MATERIALS AND VARNISHES:

*General referee:* C. S. Ladd, Food Commissioner and Chemist, Bismarck, N. D.

ACCELERATING TESTING OF PAINTS:

*Associate referee:* L. L. Carrick, Agricultural Experiment Station, Fargo, N. D.

VITAMINS:

*General referee:* E. M. Nelson, Food and Drug Adm., Washington, D. C.

VITAMIN A:

*Associate referee:* J. B. Wilkie, Food and Drug Adm., Washington, D. C.

VITAMIN D:

*Associate referee:* W. C. Russell, Agricultural Experiment Station, New Brunswick, N. J.

VITAMIN D STANDARD:

*Associate referee:*

LEATHERS AND TANNING MATERIALS:

*General referee:* I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

DISINFECTANTS:

*General referee:* C. N. Brewer, Food and Drug Adm., Washington, D. C.

NAVAL STORES:

*General referee:* F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

ROSIN:

*Associate referee:* F. P. Veitch.

TURPENTINE:

*Associate referee:* V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

RADIOACTIVITY:

*General referee:* C. H. Badger, Food and Drug Adm., Washington, D. C.

DRUGS:

*General referee:* L. E. Warren, Food and Drug Adm., Washington, D. C.

MICROCHEMICAL METHODS FOR ALKALOIDS:

*Associate referee:* C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR SYNTHETICS:

*Associate referee:* I. S. Shupe, Food and Drug Adm., Chicago, Ill.

HYPOPHOSPHITES:

*Associate referee:* H. R. Bond, Food and Drug Adm., Chicago, Ill.

**SANTONIN, PHENOLPHTHALEIN AND CALOMEL IN TABLETS:**

*Associate referee:* H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

**BENZYL COMPOUNDS:**

*Associate referee:* Solomon Reznik, Food and Drug Adm., New York City.

**HEXYLRESORCINOL:**

*Associate referee:* H. J. Fisher.

**ERGOT ALKALOIDS:**

*Associate referee:* C. K. Glycart.

**GUAIACOL:**

*Associate referee:* J. A. Batscha, Food and Drug Adm., New York City.

**RHUBARB AND RHAPONTICUM:**

*Associate referee:* A. Viehoveer, Philadelphia College of Pharmacy, Philadelphia, Pa.

**BIOLOGICAL TESTING:**

*Associate referee:* W. T. McClosky, Food and Drug Adm., Washington, D. C.

**IODINE OINTMENT:**

*Associate referee:* W. F. Reindollar, State Dept. of Health, Baltimore, Md.

**ACETPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN:**

*Associate referee:* S. M. Berman, Food and Drug Adm., Philadelphia, Pa.

**PYRIDIUM:**

*Associate referee:* H. J. Fisher.

**AMINOPHYLLINE:**

*Associate referee:* L. E. Warren.

**GUMS:**

*Associate referee:* J. H. Cannon, Food and Drug Adm., Chicago, Ill.

**CINCHOPHEN AND SODIUM BICARBONATE TABLETS:**

*Associate referee:* R. L. Vandaveer, Food and Drug Adm., Chicago, Ill.

**STABILITY OF VOLUMETRIC SOLUTIONS OF POTASSIUM IODIDE:**

*Associate referee:* S. M. Berman.

**DINITROPHENOL:**

*Associate referee:* W. F. Kunke, Food and Drug Adm., Chicago, Ill.

**THEOBROMINE CALCIUM:**

*Associate referee:* E. O. Eaton, Food and Drug Adm., San Francisco, Calif.

**CHLORBUTANOL:**

*Associate referee:* F. C. Sinton, Food and Drug Adm., New York City.

**ASPIRIN AND PHENOLPHTHALEIN MIXTURES:**

*Associate referee:* G. M. Johnson, Food and Drug Adm., Minneapolis, Minn.

**HOMATROPINE TABLETS:**

*Associate referee:* E. M. Hoshall, Food and Drug Adm., Baltimore, Md.

**DAIRY PRODUCTS:**

*General referee:* G. G. Frary, Dairy and Food Dept., Vermillion, S. D.

**BUTTER—PREPARATION OF SAMPLE AND FAT:**

*Associate referee:* C. W. Harrison, Food and Drug Adm., Minneapolis, Minn.

**CHEESE:**

*Associate referee:* C. B. Stone, Food and Drug Adm., Cincinnati, Ohio.

**MALTED MILK:**

*Associate referee:* F. Hillig, Food and Drug Adm., Washington, D. C.

**DRIED MILK:**

*Associate referee:* F. Hillig.

**MILK PROTEINS:**

*Associate referee:* W. E. Peterson, College of Agriculture, St. Paul, Minn.

**LACTOSE IN MILK:**

*Associate referee:* E. R. Garrison, University of Missouri, Columbia, Mo.

**SEDIMENT IN CREAM AND BUTTER:**

*Associate referee:* W. S. Greene, Food and Drug Adm., Washington, D. C.

**OILS, FATS AND WAXES:**

*General referee:* G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

**REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:**

*Associate referee:* Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

**THIOCYANOGEN VALUE:**

*Associate referee:* G. S. Jamieson.

**EGGS AND EGG PRODUCTS:**

*General referee:* H. A. Lepper, Food and Drug Adm., Washington, D. C.

**UNSATURATED CONSTITUENTS AND FAT:**

*Associate referee:* E. O. Haenni, Food and Drug Adm., Washington, D. C.

**GLYCEROL, SUGAR, AND ADDED SALT:**

*Associate referee:* L. C. Mitchell, U. S. Food and Drug Adm., Minneapolis, Minn.

**DETECTION OF DECOMPOSITION:**

*Associate referee:* J. Callaway, Jr., Food and Drug Adm., New York City.

**DRIED EGGS:**

*Associate referee:* F. J. McNall, Food and Drug Adm., Chicago, Ill.

**METALS IN FOODS:**

*General referee:* H. J. Wichmann, Food and Drug Adm., Washington, D. C.

**ARSENIC:**

*Associate referee:*

**COPPER:**

*Associate referee:* E. J. Coulson, Medical College of South Carolina, Charleston, S. C.

**ZINC:**

*Associate referee:* W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

**FLUORINE:**

*Associate referee:* Dan Dahle, Food and Drug Adm., Washington, D. C.

**LEAD:**

*Associate referee:* P. A. Clifford, Food and Drug Adm., Washington, D. C.

**MERCURY:**

*Associate referee:* W. O. Winkler, Food and Drug Adm., Washington, D. C.

**SELENIUM:**

*Associate referee:* R. A. Osborn, Food and Drug Adm., Washington, D. C.

**COLORING MATTERS IN FOODS:**

*General referee:* C. F. Jablonski, Food and Drug Adm., New York City.

**FRUITS AND FRUIT PRODUCTS:**

*General referee:* B. G. Hartmann, Food and Drug Adm., Washington, D. C.

**SOLUBLE SOLIDS AND EFFECT OF ACIDS ON SUGAR ON DRYING:**

*Associate referee:* C. H. Badger, Food and Drug Adm., Washington, D. C.

**ELECTROLYTIC TITRATION ACIDITY:**

*Associate referee:* R. U. Bonnar, Food and Drug Adm., Washington, D. C.

**FRUIT ACIDS:**

*Associate referee:* B. G. Hartmann.

**CANNED FOODS:**

*General referee:* V. B. Bonney, Food and Drug Adm., Washington, D. C.

**VINEGARS:**

*General referee:* A. M. Henry, Food and Drug Adm., Atlanta, Ga.

**ASH:**

*Associate referee:* H. Shuman, Food and Drug Adm., Philadelphia, Pa.

**FLAVORS AND NON-ALCOHOLIC BEVERAGES:**

*General referee:* J. B. Wilson, Food and Drug Adm., Washington, D. C.

**MEATS AND MEAT PRODUCTS:**

*General referee:* R. H. Kerr, Bureau of Animal Industry, Washington, D. C.



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### CACAO PRODUCTS:

*General referee:* W. O. Winkler, Food and Drug Adm., Washington, D. C.

### GUMS IN FOODS:

*General referee:* L. J. Cross, Dept. of Dairy Ind., Agr. College, Ithaca, N. Y.

### SPICES:

*General referee:* J. F. Clevenger, Food and Drug Adm., New York City.

### MICROBIOLOGICAL METHODS—CANNED FOODS:

*General referee:* A. C. Hunter, Food and Drug Adm., Washington, D. C.

### TREATMENT OF UNOPENED CONTAINER:

*Associate referee:* Carl Fellers, Agricultural Experiment Station, Amherst, Mass.

### SAMPLING INOCULUM:

*Associate referee:* F. W. Tanner, University of Illinois, Urbana, Ill.

### CULTURE MEDIA OF NON-ACID PRODUCTS AND SUGAR:

*Associate referee:* E. J. Cameron, National Cannery Assn., Washington, D. C.

### CULTURE MEDIA OF ACID PRODUCTS:

*Associate referee:* B. A. Linden, Food and Drug Adm., Washington, D. C.

### INCUBATION PERIODS AND TEMPERATURES FOR CULTURES:

*Associate referee:* L. H. James, Bureau of Chemistry and Soils, Washington, D. C.

### NUTS AND NUT PRODUCTS:

*General referee:* S. C. Rowe, Food and Drug Adm., Washington, D. C.

### FISH AND OTHER MARINE PRODUCTS:

*General referee:* H. D. Grigsby, Food and Drug Adm., Philadelphia, Pa.

### SUGARS AND SUGAR PRODUCTS:

*General referee:* C. A. Browne, Bureau of Chemistry and Soils, Washington, D. C.

### HONEY:

*Associate referee:* R. E. Lothrop, Bureau of Chemistry and Soils, Washington, D. C.

### MAPLE PRODUCTS:

*Associate referee:* J. F. Snell, Macdonald College, Quebec, Canada.

### DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:

*Associate referee:* C. F. Snyder, Bureau of Standards, Washington, D. C.

### POLARISCOPIC METHODS:

*Associate referee:* C. A. Browne

### CHEMICAL METHODS FOR REDUCING SUGARS:

*Associate referee:* R. F. Jackson, Bureau of Standards, Washington, D. C.

**LEAD PRECIPITATE:**

*Associate referee:* F. W. Zerban, N. Y. Sugar Trade Lab., New York City.

**WATERS, BRINE AND SALT:**

*General referee:* C. H. Badger, Food and Drug Adm., Washington, D. C.

**MINERAL SALTS AND EFFERVESCENT SALTS:**

*Associate referee:* A. E. Mix, Food and Drug Adm., Washington, D. C.

**CEREAL FOODS:**

*General referee:* V. E. Munsey, Food and Drug Adm., Washington, D. C.

**ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS:**

*Associate referee:* L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

**H-ION CONCENTRATION OF FLOUR:**

*Associate referee:* George Garnatz, The Kroger Food Foundation, Cincinnati, Ohio.

**STARCH IN FLOUR:**

*Associate referee:* V. E. Munsey.

**FLOUR-BLEACHING CHEMICALS:**

*Associate referee:* Dorothy Scott, Food and Drug Adm., New York City.

**CO<sub>2</sub> IN SELF-RISING FLOUR**

*Associate referee:*

**UNSATURATED CONSTITUENTS, BAKED AND MACARONI PRODUCTS:**

*Associate referee:* E. O. Haenni, Food and Drug Adm., Washington, D. C.

**MILK SOLIDS IN MILK BREAD:**

*Associate referee:* V. E. Munsey.

**VISCOSITY OF FLOUR:**

*Associate referee:* C. G. Harrel, Pillsbury Corporation, Minneapolis, Minn.

**COLD WATER EXTRACT FLOUR:**

*Associate referee:* F. A. Collatz, Washburn-Crosby Corporation, Minneapolis, Minn.

**ERGOT IN FLOUR:**

*Associate referee:* J. H. Cannon, Food and Drug Adm., Chicago, Ill.

**CATALASE AND PROTEOLYTIC ENZYMES:**

*Associate referee:* A. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

**COLOR IN FLOUR:**

*Associate referee:* H. K. Parker, Novadel-Agene Corporation, Newark, N. J.

**BAKING POWDERS AND BAKING CHEMICALS:**

*General referee:* G. L. Bidwell, Food and Drug Adm., Washington, D. C.

**MICROCHEMICAL METHODS:**

*Associate referee:* E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

**ALCOHOLIC BEVERAGES:**

*General referee:* J. W. Sale, Food and Drug Adm., Washington, D. C.

**BEERS:**

*Associate referee:* J. A. LeClerc, Bureau of Chemistry and Soils, Washington, D. C.

**WINES:**

*Associate referee:* B. G. Hartmann, Food and Drug Adm., Washington, D. C.

**CORDIALS AND LIQUEURS:**

*Associate referee:* J. B. Wilson, Food and Drug Adm., Washington, D. C.

**WHISKEY, RUM, AND BRANDY:**

*Associate referee:* G. F. Beyer, Alcoholic Tax Unit, Washington, D. C.

**FUEL OIL:**

*Associate referee:* Peter Valaer, Alcoholic Tax Unit, Washington, D. C.

**METHYL ALCOHOL:**

*Associate referee:* E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

**MALT BEVERAGES, EXTRACTS AND SIRUPS, AND BREWING MATERIALS:**

*Associate referee:* J. A. LeClerc.

**FOOD PRESERVATIVES:**

*General referee:* W. F. Reindollar, Bureau of Chemistry, Baltimore, Md.

**MEMBERS AND VISITORS PRESENT, 1935 MEETING**

Acton, M. G., Jr., Burroughs Wellcome and Co., Tuckahoe, N. Y.  
Adams, Georgian, Bureau of Home Economics, Washington, D. C.  
Adams, J. R., Bureau of Chemistry and Soils, Washington, D. C.  
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Allan, W. M., Raleigh, N. C.  
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Badger, Cecil H., Food and Drug Adm., Washington, D. C.  
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Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.  
Bailey, H. S., California Fruit Growers Exchange, Ontario, Calif.  
Bailey, L. H., Bureau of Chemistry and Soils, Washington, D. C.  
Baird, F. D., National Oil Products Co., Harrison, N. J.

- Baker, W. S., 177 Mills St., Boston, Mass.  
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Balthis, T. A., State Division of Chemistry, Richmond, Va.  
Barbella, N. G., Bureau of Animal Industry, Washington, D. C.  
Barnard, H. E., Farm Chemurgic Council, Dearborn, Mich.  
Bates, F., Bureau of Standards, Washington, D. C.  
Batton, H. C., Swift and Co., Baltimore, Md.  
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Berry, R. C., Department of Agriculture, Richmond, Va.  
Beyer, G. F., Treasury Bldg., Washington, D. C.  
Bidez, P. R., Auburn, Ala.  
Bidwell, G. L., Food and Drug Adm., Washington, D. C.  
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Griem, W. B., Department of Agriculture and Markets, Madison, Wis.  
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## WILEY MEMORIAL LECTURE. NO. V

### CERTAIN PRACTICAL ASPECTS OF SOIL CHEMISTRY RESEARCH\*

By WALTER HOGE MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

Each year the initial hour of the meeting of our Association is dedicated to the memory of the man who served this body, in an official capacity, for a period of 33 years. A fitting approach to a brief consideration of the present status of Soil Chemistry Research and its trend is to focus our attention upon the value of his efforts in that field of endeavor.

The scientific career of Harvey W. Wiley is an inspiration to those of us who remain to carry on. Few men in this country and in our generation have exerted so definite and beneficial an influence in the field of chemistry in its relation to agriculture. The character of the man has left an indelible impress upon many who are proud to be known as his pupils, his protégés, his disciples, his friends, his "boys." Active in this Association today are many who feel deeply grateful to him for the training and inspiration they derived from association and service with him.

Dr. Wiley was a man of dominant personality and versatile talents. He was a true scientist, an able executive, an ardent fearless advocate, and an educator in the broadest sense of that term. He tolerated no compromise in his campaign to establish firmly those principles that he conceived to be right. He feared no man, no issue, nor any consequential result of his official acts. Controversies he neither sought nor avoided. Lovable and beloved—all who knew him loved him—he nevertheless was a dauntless and persevering crusader in many of his pioneer efforts.

Rightly and justly, the memory of Dr. Wiley is enshrined in the hearts of the people of this country. To the public he was known particularly because of his championship of the cause of pure foods, and his contribution to effective legislation for that cause. His comprehensive knowledge of food chemistry and its practical aspects was universally recognized. His capacity to inspire and his ability as publicist effectively crystallized a helpful and supporting public sentiment. His clear vision and firm purpose to effectuate a new order in the food industry were responsible in large, probably largest, measure for the ultimate attainment of that order. In time, many of those who had strenuously opposed him came to realize and acknowledge that his contentions were just, justified, and mutually advantageous to the public and to industry. If his career had extended only to his attainment in the field of food chemistry, the results of his work would have constituted a worthy memorial. But Dr. Wiley was well versed in all phases of chemistry in its relation

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to agriculture. He had a profound knowledge of the literature relating to the chemistry of soils and it is in this connection that we are thinking of him today.

Soils, Volume I, of Principles and Practices of Agricultural Analysis, is a treatise that has been recognized as a most useful guide and a helpful and instructive text. Its compilation reflects a remarkable familiarity with the science of soil chemistry, as it developed in America and abroad. Dr. Wiley drew "freely on the results of experience in all countries," but he maintained that "an author is not to be blamed in first considering favorably the work of the country in which he lives," and he paid tribute to the help obtained by him from his confreres, Hilgard, Merrill, Osborne, and Whitney. In his final edition of Volume I, he transferred to Volume II certain contents that related "more to fertilizers than to soils"; included the "latest improved methods—insofar as they are based on new principles or have secured better results"; and retained "many of the older methods long out of vogue—because in them are found the beginnings of fundamental procedures which seem to unify the processes of analysis and render more intelligible the modern methods." Truly an appreciation of the new, mingled with a justified loyalty to the old.

Wiley's approach to the problem of soils was a remarkable balance between full recognition of the bearing and importance of the related sciences and comprehension of the economics of soil fertility. In considering edaphology, data of cosmogony were used; an appeal was made to the science of geology, to physical geography and glaciation, to mineralogy; to the influence of meteorology upon the formation and transposition of soil material; to the importance of bacteriology; and to consideration of environmental influences involved in ecology and climatology. Wiley subscribed to the fact that "the soil cannot be regarded as dead matter, but as a living organism exhibiting many most remarkable biological phenomena." The dominant consideration, however, was based upon "discussion of those principles resting on chemical and physical data with the inclusion only of reference to the related sciences, which serve to elucidate the fundamental principles of the study and technical methods of approved processes of analysis." Pointing out further that "in economics we seek the solutions of all problems determining successful agriculture, in particular relative to soil fertility and a favorable climate," Dr. Wiley concluded, "It is evident that a complete work on soils could only be written by the collaboration of experts in all the above-named branches of science." But, even with recognition of the importance of the related biological sciences, and the so-called new concepts, the approach stressed in Dr. Wiley's period of active participation in soil research was, in the main, based upon a geochemical and mineralogical background.

In excellent American texts, such as those of Johnson, Storer, Hilgard, King, Lyon, and Buckman, the embrative science of pedology is well

presented, but Volume I of Wiley's Principles and Practice of Agricultural Analysis was primarily intended to be a comprehensive compilation of chemical methods and their adaptations. Dr. Wiley's fundamental training in chemistry and his comprehensive knowledge of analytical technic were such that his especial interest lay in the development of quantitative procedures that could be used to obtain information which could be utilized in the formulation of a scientific philosophy. His appreciation of the importance of analytical methods was reflected in the fact that at the inception of the organization of this Association the perfecting of methods for soil analysis was included as a major objective.

Dr. Wiley was not a patriarch in agricultural chemistry or in soil chemistry. The promulgation by Liebig of his philosophy of the rôle of soil chemistry caused him to be considered as the founder of that science, just as the treatise "Grundsätze der Agrikulturchemie" gave to Schübler the title of father of agricultural physics. But it soon appeared that the mere analysis of a soil could not be used as a yard-stick for measuring fertility. As Hilgard has pointed out, in discussing Liebig's contribution, when the absolute chemical analysis of long-cultivated soils failed to give the anticipated correlation between soil composition and plant response, "few thought it worth while to occupy their time in chemical soil analysis."

A study of the trend of analytical procedures brings out the fact that the early approach considered in particular the relation of soil to parent rock. Hilgard pointed out that this relationship is most effectively developed through the analysis of virgin soils, rather than soils altered by cultivation. In time it was recognized that a more comprehensive analytical attack was necessary to furnish adequate information as to the chemical properties of a soil. The methods that had been evolved for the analysis of rocks were adapted to the bulk analysis of soil melts. By means of "bulk" or "ultimate" analyses, the body of the soil, including "soil minerals in their original form," was completely disintegrated by fusion to permit of determination of the dominant component silica and to furnish a solution of the elements of recognized importance. This type of analysis was supplemented by acid extractions to determine the rock-derived "reserve" components, as a measure of "productive capacity," or "permanent value." Likewise, King followed in the paths of Ulbricht and Schultze and utilized aqueous extractions to remove those nutrients present and available for immediate assimilation by plants. The use of a strong acid solvent for the extraction of the "reserve" bases and the amphoteric elements was developed particularly by Owen, Robert Peter, Loughbridge, Hilgard, and Kedzie, to whom we are indebted for the use of the method that stipulates the constant boiling point hydrochloric acid of 1.115 sp. gr. The immediate productiveness, the "Dungerzustand" of the German agricultural chemists was studied by means of milder

solvents. In parallel with the contributions of Dyer and Maxwell, who used the weak citric acid of 2 per cent concentration and aspartic acid, respectively, as solvents to measure the available supply of  $P_2O_5$ , the use of a dilute solution of a mineral acid, such as N/5 nitric, was advocated by Peter and by Fraps as a measure of availability of the P and K content of soils. In the study of the water-soluble components of soils of the humid regions, it was necessary to develop and adapt both the mechanics and chemistry of procedures to permit of the determination of even the minutest quantities of the desired solutes. To fill this need came that most useful manual "Colorimetric, Titration and Turbidity Methods" by Schreiner and Failyer, and the "Schreiner Colorimeter."

Many of the earlier workers in agricultural chemistry were unable to give their full time and effort to research. Heavy undergraduate teaching duties, extension activities, fertilizer control, and other responsibilities precluded "continuity of thought and effort" in the field of investigation. But, with the increase in facilities in the Agricultural Experiment Stations and the expansion of a program of soil research within the U. S. Department of Agriculture, an impetus was given to research in soil chemistry. With the increase in personnel that was made possible by the advent of appropriations, derived first from the Adams Act and later from the Purnell funds, intensive research into fundamental problems was greatly accelerated and extended. This influx of personnel of specialized training in physical, organic, and biological chemistry was made possible by the remarkable growth in curricula that had been developed in the graduate schools and colleges of this country. Many, if not most, of the earlier leaders had received their graduate training abroad, but the reverse situation now prevails. Adaptation of the tools of the several specialized fields of chemistry to the problems of soil chemistry was responsible for new approaches and concepts and novel methods of attack. It was pointed out by Sir John Russell in his presidential address at the Third International Congress of Soil Science that "not until the great awakening of science after the war were there either men or enough resources to enable any important number of them to devote themselves exclusively to soil investigations." It should be noted, however, that some of the supposedly new lines of attack were only modifications of lines of procedure that had been utilized by the pioneer workers who labored under disadvantages of paucity of equipment, meager funds for maintenance, and inadequate personnel, and in particular, unperfected analytical methods.

Acknowledgment should be made of one factor that has served to stimulate research and development of analytical procedures and to insure the presentation of experimental data and conclusions. For many years the channels for adequate publication of experimental results and and new methods were, in the main, through bulletins and in the limited issues of annual reports in which many valuable contributions were prac-



tically hidden. A useful and dependable method would be developed without becoming widely known. There were, of course, occasional contributions carrying particular academic interest that would permit of publication in certain of the journals devoted either to pure science or industrial chemistry. The inadequacy and handicap of this situation was recognized, and steps were taken to alleviate the need. The field of soil chemistry owes a particular debt to Jacob G. Lipman for his vision, sagacity, and zeal in creating and maintaining that journal of world-wide recognition, *Soil Science*, the columns of which have carried numerous contributions that have been helpful in perfecting analytical methods. The more recent workers in soil chemistry have also had access to the columns of the *Journal of the American Society of Agronomy*, the *Journal of this Association*, *Journal of Agricultural Research*, *Industrial and Engineering Chemistry*, foreign journals including those of the *International Society of Soil Science*, and certain "trade" publications as outlets for publication of analytical procedures. Our pioneer workers were not so fortunate.

This Association is especially interested in the development of methods and in their influence upon both academic and practical phases of soil chemistry. Along with perfection and refinement of analytical methods came findings that were used as the basis for either new or revived schools of thought. The older members of this body will remember the occasion some 30 years ago when the then president of this Association, a distinguished and useful scientist, militantly presented his philosophy of "Soil Fertility and Permanent Agriculture." That presidential address was deemed by some to be an unwarranted attack upon a divergent philosophy advocated by the Chief of the Federal Bureau of Soils. At that time, publication of both the proceedings and the methods of this Association was provided through the medium of Federal bulletins, and the then Secretary of Agriculture would not permit that presidential address to go out in the usual channel. The dissertation did appear, however, as a Station bulletin. The two divergent concepts, one advocated by a prominent, forceful, and earnest Station chemist, the other advocated with equal earnestness and conviction by an able scientist with the collaboration of an associate, a vigorous and modern scholar, were evolved from data obtained, in the main, by two different types of analytical attack. Deplorable as were the unfortunate bitterness and personal animus engendered, that controversy served at least one happy and beneficial result, namely, the stimulation of interest in and an appreciation of the importance of soil chemistry and soil fertility.

The continued use of land under improper cultural conditions, destructive single-cropping systems, and the cumulative effects of injudicious fertilizer usage and attendant depletion of soil components, have resulted in new and pressing problems in fertility. The marked depletion in certain

components of many soils in the regions where fertilizers have long been used intensively and the advent of indications of depletion in areas for which it was contended by many that fertilizers would probably never be required have caused the science of soil chemistry to become more and more interwoven with the chemistry of fertilizers. We now recognize that the injudicious handling of soils has resulted in residual effects that are not constant for all types of soil. Thanks to developments in analytical procedure, it is now possible to predetermine within reasonable accuracy the variant reactions that ensue between certain types of soil and certain forms of fertilizers. It is essential to know not only the amount and availability of the single components of fertilizers and their immediate effect upon plant growth but also what transpires when different forms of plant food are mixed together before the mixtures are incorporated with the soil. This is particularly true since the advent of the many new fertilizer products.

The marked increase in the necessary use of various insecticides has also injected new problems relating to the effects induced by their residues. Moreover, it has been determined that serious problems may arise from the cumulative effects of increments of certain toxic elements, such as boron, that are contained in irrigation waters. But the most serious problem to tax the ingenuity of the soil chemist and the agronomist is the one of exposures of new soil horizons by erosion, in its various phases. In the rehabilitation of subsoil into a cultural medium of true soil or "soil material," we have the advantage of knowing from certain types of attack that the two systems, soil and subsoil, have certain fundamental and inherent properties of variant if not divergent nature.

In the development of soil-fertilizer chemistry to its present status, there have occurred periods when certain phases of research and analytical methods for their promotion were stressed more or less intensively. The relation of chemical and biochemical methods to these topics and periods show, more and more, a trend toward practical application. This trend can be pointed out only inadequately within the time available for the present discussion, but at least a few points may be brought out to demonstrate conclusively the fact that soil chemistry offers a fertile field for the trained investigator and affords him opportunity for constructive, useful, and practical service. Although the need for findings capable of practical interpretation is an imperative one, it is essential that we retain a love for and an appreciation of research of academic nature, even when no imminent uses of anticipated results are apparent. It cannot be foretold what application today's academic disclosure will have tomorrow.

Discovery of new factors and development of new concepts have gone hand in hand with evolution and perfection of methods. Frequently, the attainment of an anticipated objective has been delayed because of lack of an adequate and dependable analytical technic. As a sequence to

qualitative tests, such as the litmus paper test for soil reaction, have come quantitative procedures for the determination of the amount of lime that a soil can absorb, "active acidity" as distinguished from "negative" acidity, or the analogous "immediate" and "continuous" lime requirement, organic acids as set forth by Schreiner and Shorey, and concentration of hydrogen ions as expressed by the Sørensen values of  $pH$ , and their unity equivalents given in our *Methods of Analysis*. Numerous methods, such as those of Veitch, Hopkins, Tacke, Daikuhara, Truog, Hissink, Bizzell-Lyon, Hutchinson, Conner, Loew, Carr, Rice-Osugi, Holleman, Immendorf, Jones, and Bradfield, to mention a few, have been used to obtain variant values. These several procedures were based upon varying methods of approach—direct treatment and reaction with alkaline solutions, exchange reactions, and evolutions of  $CO_2$  from disintegration of added carbonates. In some cases the method may have been developed subsequent to the concept that prompted the technic, whereas in other cases the results obtained by the procedure were used to develop the concept. After years, the old and logical principle of equilibrium utilized by Veitch to determine the immediate capacity of a soil to react with lime is still recognized as fundamentally sound and the main deterrent against its use is the multiplicity of tests necessary when working with soils of unknown history. Likewise, the method of Jones, which is based upon ionic replacement through treatment of a soil with a solution of calcium acetate to engender titratable acidity, is still highly regarded. These two contributions came from two workers who have made other valuable contributions to the work of this Association.

Such studies have resulted in much useful information as to the causes for the development of soil acidity, the nature of the resultant acidic complex, and the variant effects of different fertilizers upon either the advent of that condition, or its antithesis. In South Carolina, Cooper utilized emergency employment funds most effectively to obtain field samples that permitted a detailed record of the reaction of the soils of that State. Following the growth of the synthetic nitrogen industry, which has resulted in such an increase in the production and use of those forms of nitrogen that engender soil acidity, which can be designated also as a deficiency of exchangeable bases, the subject of soil reaction has become a problem of recognized importance, in relation not only to the quantity, form, and availability of the alkaline earths, but also to the supply of the amphoteric elements. Following the researches at the Alabama, Tennessee, and West Virginia Stations and in the Bureau of Chemistry and Soils, we now have, thanks to Pierre, a most promising procedure for the evaluation of the potential acidity or the potential alkalinity of a commercial fertilizer. It is of especial interest to note how favorably the fertilizer industry has reacted to this development and to the correlary problem now under referee study, namely, methods for determining the fate of magnesian supplements.

Another fruitful field of endeavor is that of the variant reactions that ensue between the several phosphates and soils of different degrees of acidity and variable quantities of the amphoteric elements. There is an especial need for analytical procedures that will throw light upon the rôle of colloidal silica in the assimilation and utilization of phosphates by growing plants. The classic studies of Mattson and his associates have been most helpful in affording an understanding of the fate of added phosphates. The contributions by Breazeale, Gile, Ellett and Hill, Gordon, Shedd, Truog, Midgely, Magistad, Parker and Tidmore, Conner, and others have done much to clarify the phosphate problem. From the investigations of Pierre, we know that an acidic phosphate may decrease the pH value of one acid soil and increase that value in another, and from this work and that of Conner we have the conclusion that the more acid the soil the more basic and less soluble in water should be the added phosphate and vice versa. In one Station an effort was made to increase the mobility of  $P_2O_5$  by means of magnesium phosphate, and the work of another Station led to the obtaining of a patent upon the principle of adding organic un-ionized forms of phosphates that insure a greater migration or dissemination and delayed fixation of the  $P_2O_5$ , so that the roots of deep-rooted plants can develop in zones enriched in phosphates.

Lysimetry has been utilized to obtain both a better understanding of the fundamental reactions that transpire in a soil system and also information directly adaptable to practice. Lysimeter installations of various types and for different objectives have been made in Hawaii, at the Florida, Indiana, Geneva, Cornell, Missouri, Oregon, Illinois, New Jersey, Virginia, Kentucky, Connecticut, North Carolina, Missouri, and Tennessee Stations and at the Federal Sand Hill, South Carolina, Station. The late Doctor E. W. Allen expressed the belief that the equipment of an Agricultural Experiment Station was incomplete in the absence of such an installation.

This type of investigation has greatly augmented our knowledge concerning the seasonal outgo of nutrients; the influence of soil depth and variations in the behavior of different types of subsoil; the conservation of added anions and cations; interchange reactions induced in the subsoil by leachings from the surface soil, in contrast to "reciprocal repression" induced in the zone of incorporation; the relation of rate, form, fineness, and zone of incorporation of different liming materials upon the behavior of calcium, magnesium, and both native and added supplies of potassium; the lime-magnesia relationship to sulfofication and sulfate conservation; the divergent effects of lime and magnesia upon the natural and added fluorine content of the soil; the fate of barium additions; and the effect of season of incorporation upon the variant behavior of different nitrogenous materials. With the many questions that may be expected to arise because of the make-up and chemical characteristics of certain of the fertilizer concentrates, there will be an increasing need for this type

of experimentation. It is readily apparent that lysimetry can be utilized advantageously for guidance in the rehabilitation of seriously eroded soils in the humid region and to acquire information as to the effects that various types of legume and non-legume vegetation and their residues exert upon conservation of plant nutrients.

The importance of certain of the so-called less abundant elements has come to the front during recent years, and this Association has been studying methods for the determination of such elements. It is known that some of these elements exert either a benign or a deleterious effect as determined by form, amount, and soil type and reaction. The activities of arsenic, zinc, boron, copper, barium, fluorine, iodine, manganese, iron, and selenium have been studied and the practical relationships have been recognized. Recently the Chilean Nitrate Educational Bureau, Inc., sponsored a comprehensive compilation of the literature relating to "the minor plant nutrients." The recent studies of Byers and associates of the Bureau of Chemistry and Soils and by Franke of the South Dakota Station relative to the localized importance of abnormal quantities of selenium and the ameliorative effect of sulfur additions in such cases is an example to the point. It would be most illuminating if it were possible to obtain for the dominant soil types, at least, complete inventories such as those of Hillebrand, and those of Clarke, which show the elements present in the parent rock, and such as the classic contribution made by Thomas of the Pennsylvania Station.

In recent years the field of base interchange has been explored by many investigators in this country and abroad, and a number of analytical procedures have been evolved. The beginning of research in this useful field can be dated from 1849, as a result of the classic replacement studies of Way, and impetus has been given to the subject through practical problems such as those encountered in the reclamation of alkaline and saline soils. In the great Zuider Zee project of the Netherlands, whereby many thousands of acres have been reclaimed from the ocean floor, the classical base-exchange researches of Hissink have been invaluable in affording understanding as to the transition of sodium clays into calcium complexes. In this country, the base exchange researches of Kelley and his colleagues in California have been of outstanding value when interpreted into practices that remove the toxic accumulation of salines and carbonates. This is true also of the fundamental studies of Burgess, McGeorge, Breazeale, and Magistad at the Arizona Station in relation to the paucity of carbon dioxide as influencing the availability of phosphates and the repressive effect of alkalinity upon potash solubility. These and dialytic studies, such as those of Bradfield and of Wilson, have led to a better understanding of the behavior of the soil bases that are held in the non-mineral state and the replenishment of those exchangeable bases by the hydrolytic breakdown of the mineral reserves and also the interchange reactions induced by the soluble fertilizer salts.

Within recent years, however, there has come an insistent demand for methods that can be used economically and rapidly to determine the need for the ordinary fertilizer elements, at least, and for specific and limited areas. Laymen have been unable to understand why the soil chemist cannot analyze a soil, determine all deficiencies, and prescribe panacea. If it were feasible so to do, there would be created a useful service and one well calculated to produce on the part of the public a friendly attitude toward and support for research programs. Station workers are accustomed to the requests from individuals and corporate bodies and county agents for soil analyses. Such requests are frequently based on the assumption that those who make the requests can supply the correct interpretation of the analytical results. Associations of growers also request conferences and seminars on the problems of infertile soils and the economic use of fertilizers. In Germany, in particular, soil chemistry laboratories are supported, in part, by the income from a nominal fee charged by the laboratories for chemical tests of submitted samples.

In this country, field-test methods such as those of Hoffer and those subsequently developed by others, particularly at the Indiana Station, have been used to test the tissues of field crops for specific nutrient deficiencies. Intelligent observations have been responsible for means of ready recognition of symptoms registered by plant tissues in soils that have developed marked nutritional deficiencies, especially in the case of magnesium, calcium, and potassium. In several Stations, Michigan, Illinois, Wisconsin, and Indiana, qualitative methods have been developed for the testing of soils in the field to determine the response to be anticipated from specific fertilizer treatments. If such methods could be used, in conjunction with the simple tests for soil reaction, by those who are engaged in soil surveys, the value of such surveys would be enhanced greatly. The opportunity for such correlation is particularly timely in those areas where collaborative efforts between Federal agencies and Experiment Stations are being concentrated to obviate erosion through soil restoration.

In the search for quick indicative methods, ones more rapid than pot trials, investigators here and abroad have developed such methods as the biological culture procedure of Nicklas, which measures the response shown by *Aspergillus Niger* to manurial treatments, the azotobacter plaque method of Winogradsky, as modified by Sackett, and the *Cunninghamella* plaque technic developed at the Wisconsin Station. In this country the method that has been utilized most extensively is probably the one developed by Neubauer, who contends that his method presents an answer to the objection raised against the use of dilute solvents intended to represent the dissolving action of plant root secretions. Since, as Hilgard states, "We cannot imitate plant root action," Neubauer adopted the principle of measuring the supplies of available nutrients,

through the assimilating capacity of growing plants. A standard seed supply of rye, and a plant tolerant of a considerable range in pH, moisture, humidity, and temperature control for a standard period are requisite conditions. Neubauer postulated that the mass of roots developed during the 17-day growing period will exert a feeding power that will register the quantity of available nutrients in the soil medium and the response to additive materials, when the composition of the plant ash is determined. It will be recalled that his method was used to advantage by Kraybill and Thornton, and by Ross in their collaborative studies to show the availability of the "citrate-insoluble"  $P_2O_5$  engendered in fertilizer mixtures in contrast to that carried by fluorapatite and in measuring the availability of the potash that is rendered water-insoluble in the analytical charge when the technic of the official procedure is followed. When it can be shown that the satisfactory correlation between the results obtained by this rapid method and the findings from orthodox field experimentation in the State of Indiana will obtain also in other humid sections, the Neubauer procedure will be used even more extensively.

The curse of the fertilizer-consuming South has been erosion, both gully and sheet. The ultimate results—devastation, soil abandonment, loss of taxable property, and to many the heritage of abject poverty—failed to crystallize public conscience. The conditions that now prevail require speedy remedial action. The convincing data advanced by Bennett, to whom the nation is indebted for his long-maintained recognition of and fight against erosion, together with the graphic and pictorial showings in magazine articles have caused the public to become erosion-conscious. But, with the coming of governmental activities directed toward flood control of navigable streams and the necessity for protection to obviate the silting that would destroy the usefulness of the dams provided for such control and conservation of flood waters, the public has been educated to the necessity for the economic control of a national evil and peril that, in general, had been either ignored or tolerated as a necessary evil.

In the rehabilitation of the water-eroded eastern areas by reforestation, afforestation, terracing, withdrawals of slopes from cultural cropping, and other methods, and in the bringing back of grass cover to the wind-eroded areas west of the Mississippi, it is essential to obtain dependable pilot information concerning the inherent properties of the newly-exposed horizons and their fertilizer requirements. In his recent comprehensive treatise, "The Land of Our Possession," President Bowman of Johns Hopkins University, states, "After the fields have had their top soils blown off, we enter a long period of both waiting and experimentation to see if the grass will grow again. . . ." He inquires as to the kind of society that may be expected in "the high-risk areas of the Western Great

Plains" and he quotes Bacon to the effect that in troublesome times one of the first remedies is "the improvement and husbanding of the soil."

Furthermore, it may be expected that the intensive program of fertilizer research, both governmental and commercial, will develop new types and combinations of fertilizers, the tonnage production of which cannot proceed, in some instances, until dependable data as to their value have been acquired. The soil and fertilizer chemists will be expected to adapt the present methods and to devise new ones that will assure the determination of the chemical composition and properties of such new forms.

In a consolidation of the program for soil rehabilitation the contributions of the soil survey specialists, the agronomists, and the soil chemists will be required. The soil chemists of this country will accept the challenge that they contribute to the practical demands of the present emergency.

During the recent years the trend in soil chemistry research has been more and more toward the solution of problems, both extant and anticipated. Although it is undoubtedly true that such research has been expedited and intensified, particularly during the last two or three decades, it is nevertheless true that the present structure of soil chemistry rests firmly upon the foundation erected by those resourceful pioneers of whom Harvey W. Wiley was one.



## PRESIDENT'S ADDRESS\*

### RESEARCH AND OUR FOOD INDUSTRIES

By F. C. BLANCK (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.)

According to the 1933 census of manufactures issued by the United States Bureau of the Census, the manufacture of foods and food products ranks first among the various industry groups in the United States, with a total value, even in this period of depression, of slightly over six and one-half billion dollars. Of this total value more than one-third was added by manufacture.

I am deeply conscious of the difficulty of presenting adequately to this Association a subject of such vast magnitude. Our members are all chemists, and yet our fields of work are so varied that many of us have only a very general knowledge of the part which science plays in our food industries. Many of those whose present activities are in the food field are concerned primarily with analytical problems, therefore it may be of some interest to point out the vital part which science, and chemistry in particular, has played in bringing our food industries to their present state of efficiency. Obviously the field is so large that, with our limitation on time, it will only be possible to indicate some of the more outstanding contributions to certain of our major food groups, and the vitally important rôle which research has played in their development, and to suggest some problems which still await solution, the answer to which will result in a definite saving to the consumer and producer by the elimination or retardation of the losses that now occur. The broad application of scientific and chemical research to the problems of the food industries is of the greatest importance to the world at large and to all mankind.

A study of the commercial methods now employed in a number of food industries in the preparation of their products indicates clearly the outstanding importance of scientific research to present-day practice. While many of these manufacturing operations received their initial impetus from the hard school of practical experience, scientific research showed the foundation on which this experience was built, and it furnished the new information which promptly became the basis for practical plant operation. One of the outstanding illustrations of this type of development is furnished by the canning industry, which is one of our major food groups. In an address a few years ago, under the title "Research from the Food Manufacturer's Standpoint," Mr. Richard Dickinson, former Chairman of the Research Committee and ex-President of the National Cannery Association, said, "The canning of food has grown in one generation from a few scattered shops, run by rule of

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\* Presented Tuesday afternoon, November 12, 1935.

thumb and guesswork, to become one of the leading industries of the country; and the largest factor in that growth was the application of research in chemistry and bacteriology." Later in referring to fundamental research, he said, "This type of work, slow and unsatisfactory as it often appears, is most important as it is the foundation for all the specific work which produces the direct, practical results that are of immediate value. It is not as foolish to attempt to raise a great beautiful building on an insufficient foundation as to expect successful research without having an adequate basis of fundamental work from which to develop it." In this great industry it was early recognized that it is only by scientific research that such fundamental problems as heat penetration in the tin container, time and temperature factors in the distribution and viability of microorganisms, and the relation of acidity to process times and temperatures, can be solved. These problems were attacked systematically and with painstaking care, and as a result these factors are now determined with scientific exactness. Guesswork or the trial and error method is a thing of the past. Later, with the rapid development of our knowledge of vitamins, it became imperative to know whether or not the canning process impaired the vitamin value of foods. Here again the carefully conducted experiments of Kohman, Eddy and their co-workers through a period of years, established the fact that there is no impairment in the vitamin value of foods preserved by commercial methods in hermetically sealed containers. Another problem affecting this industry, the black discoloration of canned corn and certain shellfish, was finally solved by the incorporation of very small amounts of insoluble zinc oxide in the enamel lining of the can. Although this solution seems rather simple the search for it continued more than 15 years, and it required the services of many chemists and the expenditure of hundreds of thousands of dollars. It is certainly no exaggeration to state that the canning industry owes its present high state of efficiency and the safety, high quality, and general acceptability of canned foods to the application of principles developed primarily by scientific research.

Meat packing is the largest food industry and its progress illustrates a real romance of science. At the present time its development is completely interwoven with physical and chemical science. The research work has been concerned not merely with the fundamental problems involved in chilling, ripening, curing, cooking, canning, and the manufacture of sausage, lard, fats and oils, hides, skins and pelts, which may be regarded as direct products of this industry, but it includes the application of chemistry and other sciences, which has made possible the development of an amazing number of by-products. More than 140 of such products are now manufactured commercially. They include such articles as alkalis and washing powders, ammonia, wool scourings (which are used as a base for cosmetics), epinephrine and tallow, beef peptone and pituitary

extract, billiard balls and soap, buttons and shoes, case hardening materials and sandpaper, casings, ligatures and violin strings, diastase, greases and insulin, digestive ferments, lecithin and neat's-foot oil, candles and cosmetics.

Baking is another of our major industries, and even in this depression period it reached a volume of nearly one billion dollars. Here again, scientific research has played a leading part. Our knowledge of the chemistry of wheat flour, of the conditions producing the maximum efficiency of yeast in fermentation, and of the stimulation of yeast growth, has been far reaching in its application in this field. Notwithstanding the progress that has been made, much remains to be learned of the physical-chemical changes that occur both in the dough stage and in the finished loaf. No single satisfactory test has yet been developed for determining the baking value of wheat flours, and the great problem of the staling of bakery products still remains unsolved.

In the field of jams, jellies, and preserves, the scientific starting point was the pioneer work on the pectic substances published by Payen in 1824 and by Braconnot in 1825. Since that date, the classical researches of Willstätter, Euler, Sucharipa, and Carré and Haines, have gone far in elucidating the complex chemical nature of these substances. The work of Nelson has given us our present knowledge of the various acids naturally occurring in our fruits. Tarr and his co-workers, Meyer and Baker, by a series of classical researches on the acid-sugar-pectin ratio established the scientific basis on which present-day jelly manufacture is founded.

The immense field of dairy products and by-products owes its progress primarily to scientific research. Since milk and other dairy products are among our most important foods and have a very definite health aspect due to the possibility of contamination, it is obvious that this field should early have received the benefit of most careful scientific research. This is particularly true in the field of milk pasteurization in relation to the effect of this process on the nutritive value of the product, and especially in relation to the destruction of pathogenic organisms and the organisms involved in its spoilage. More recent study has definitely shown how to enrich milk in vitamin D and certain inorganic chemical elements of nutritional importance. The technology of manufacturing dairy products such as cheese, butter, ice cream, and evaporated and dried milk owes its present high state of efficiency to scientific research. Such old established food industries as the confectionery, sugar, milling, beverage, and vegetable oil, also owe a constantly increasing debt to scientific research.

Among our newer food industries, the birth and development of the frozen pack industry as a means of preserving meats, sea food, fruits, and vegetables is truly a romance of science and invention. The development of this new method was made possible by fundamental research

involving the study of biochemical, physiological, and physical changes taking place at low temperatures. The success that has already followed this new industry is stimulating further research on the behavior of microorganisms and enzymes at below-freezing temperatures and particularly their behavior during varying periods of storage at temperatures higher than the initial freezing temperature. It is safe to predict that the future development of this new industry will be absolutely dependent on the progress of scientific research on the many fundamental questions involved.

No discussions of the relation of research to the food industries could overlook the marvelous progress that has been made in the field of food containers as a result of scientific research. Such materials as cellophane, glassine, wax paper, vegetable parchment, paper cups and sacks, and metal foil have demonstrated beyond any doubt their rôle in bringing our manufactured and prepared foods to the ultimate consumer in the form and condition in which they were prepared.

The food field is practically endless in the opportunities it affords for research of scientific and practical interest. Rancidity—its nature, cause, and prevention—is still one of the outstanding food problems that merits any amount of new and constructive thought. The color and flavor of natural foods, such as fruits, is a field in which comparatively little has been reported. The economic importance of such knowledge is exceedingly great when one considers the various types of manufactured food products which are made from such fruits. There is a distinct lack of knowledge of the ash constituents of our American foods, particularly so in the light of the improvement in analytical methods brought out in the last ten or more years. Our knowledge of the nature of the enzymes present in fruits and vegetables and of the rôle they play in deterioration and spoilage is meager, and much work is needed in this field. Food spoilage and deterioration offers a splendid field of work for the collaborative efforts of the research chemist and biologist. The waste or refuse material incident to food packing and distribution offers a constant challenge to the chemist to devise profitable methods for its utilization. The effect of light on the composition, color, and even physical consistency of manufactured foods offers a tremendously fruitful field of research, the surface of which has hardly been scratched.

It is no exaggeration to state that no food industry can expect to exist, much less to progress, without the aid of scientific research. Many of our food industries recognize this and admit their absolute dependence on scientific research for progress. Their attitude is one of definite encouragement expressed in such concrete forms as the financing of individual group research, the encouragement of food group association research, the development of a research fellowship program, and the active cooperation with the Federal government on problems of general

interest to the particular food industry involved. This situation is forcefully stated in the following timely and thought-stimulating editorial taken from a recent issue of "Nature":

"Research means more than the invention of some ingenious mechanical device which captures the imagination, or alleviates an unwelcome personal exertion; it is not served by romancing Press 'stories' in which a slender basis of fact is made the theme of a sensational announcement. Whether its achievements intrigue the public, or whether they can be appreciated only by technicians, the simple fact is that scientific research has proved itself to be one of the pillars of modern competitive industry. What the ordinary citizen so often fails to realize is that part which fundamental scientific research (exemplified by that performed at the universities) and applied technical research (exemplified by that performed by the industries themselves and by governmental agencies) is *already* playing in maintaining him with some degree of stability and comfort in that state of life which he has reached. Nor does he properly appreciate the crash—industrial, political, and social—which would necessarily follow the neglect of opportunities to acquire new knowledge of material resources and new power to use them."

In conclusion may I paraphrase a statement made nearly 40 years ago by one of our illustrious founders and ex-Presidents, Dr. H. W. Wiley:

To him who writes the story of the progress of our food industries as influenced by chemical and scientific research during the next half century may come a feeling of pity for the ignorance which now surrounds us; but he will at least accord to our workers the merit of being emancipated from the slavery of opinion and the worship of authority. He will certainly say they were patient, industrious, and truth loving. To the leaders of progress for the next half century we commit our unfinished work, confident of their integrity and hopeful of the good which they will bring to mankind.

## ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

### THIRD DAY

#### WEDNESDAY—AFTERNOON SESSION

##### REPORT OF EDITORIAL BOARD

The work of the Association has proceeded along the usual lines. This, as you know, is the fifth year of our program in regard to the revision of *Methods of Analysis*, since it is revised every five years. Some of the plans discussed at the last meeting did not materialize during the past year for various reasons.

At the meeting of the Executive Committee, held at the Cosmos Club, it was decided that the 750 copies of *Methods of Analysis* which are now unsold may be sold for \$2.50 each, beginning December 1. The question was raised as to the possibility of these books being of special value to students in cases where it is not so important that they have the latest revision. Therefore, if any of you are interested in having these books distributed in schools, I shall be glad to have you so indicate.

The reports for the Editorial Board will be made as customary by the chairmen of the several committees. The first committee report, that of *The Journal*, will be given by Mr. H. A. Lepper. *The Journal* has had a very successful year under the management of this committee.

W. W. SKINNER

Approved.

##### REPORT OF EDITORIAL COMMITTEE OF THE JOURNAL

This year *The Journal* progressed in two ways. The reversal of the trend toward diminishing subscriptions, noted last year, has continued. New subscriptions number 58. To offset this, however, cancellations totaled 48, but a number of these were subscriptions due for the past two or three years, which were cancelled after repeated billing failed to bring payment for any year. Further revision of the list is contemplated at the beginning of the next volume.

The volume just completed includes 661 pages, the largest since Volume VIII, which covered a year and a half, with the exception of Volume XV of 1932. This growth was due to the increase in contributed papers, to which 243 pages were allotted, as compared with the yearly

average of the past six years of 165 pages. The contributed articles dealt with subjects in practically all fields of the Association's activities, including the ones most recently added to the studies of referees, such as enzymes, microchemical methods, fish products, and vitamins.

The desire of the Association to allot more space to contributed articles has been fulfilled. This advance should be maintained, as it is evident that continued success of *The Journal* will be closely associated with the number of contributed papers of high quality and broad scope that are published. The Association justly looks to its members to increase the prestige now enjoyed by its *Journal*. It is not inappropriate at this time to remind members having original work to report to give first consideration to this, *Their Journal*, as a medium of publication. All papers dealing with methods should certainly be submitted and papers treating of subjects of broader interest to workers in the special fields to which *The Journal* is devoted should not be excluded.

The decision of last year to devote more space to book reviews has been brought to the attention of all publishers of scientific books in this country and to some importers of foreign books by personal letter from the Editorial Committee. They were invited to submit for review copies of new books on analysis or those dealing with the chemistry and technology of the products of agriculture in its broader aspects. As a result numerous inquiries and some books have been received. The next volume of *The Journal* should show an increase in book reviews.

A few years ago it was decided to feature special articles from time to time on critical studies of methods relating to specific subjects. As yet no paper of this character has been contributed. It is felt that this feature should be developed.

Complaints have been received that the Association is not as prompt as is desired in publishing the changes in the methods that are made at the annual meeting. Beginning with the next volume an attempt will be made to issue the first number carrying the report on changes in the methods January 1, the other numbers to follow in April, August, and October. Because of the extra work involved in the Editorial Office in connection with the revision of the *Book of Methods* it may not be possible to follow this program completely this year. However, the first number of the forthcoming volume will be issued as near to January 1 as possible, and the program will be put into effect with the remaining numbers.

H. A. LEPPER

Approved.

#### REPORT OF EDITORIAL COMMITTEE OF METHODS OF ANALYSIS

The plan adopted by the Association and followed in recent years whereby all additions to, and changes in, *Methods of Analysis* are summarized in the first issue of our *Journal* following each annual meeting

has greatly facilitated the work of compiling the revision of our *Book of Methods* at the five-year intervals. However, the task is still one which requires much time and attention.

The following basic plan of procedure has been followed by the Committee:

(1) Revision of all chapters by the Committee to include the changes made since the last revision.

(2) Submission of the several chapters to referees and others best qualified to give detailed and constructive criticism of the subjects involved. As an aid to reviewers of chapters a schedule of general points to be observed was drawn up by the Committee and submitted to each reviewer with the chapter sent to him.

(3) Return of the chapters to the chairman with comments and suggestions by the referees and others.

(4) Consideration of comments and suggestions by the Committee with further correspondence where necessary to clear up difficult or doubtful points. Some chapters have been returned for a second review.

A summary of progress, made as of October 4th showed that—

(1) All chapters for which there is subject material have been revised in the Committee and transmitted to the respective referees and/or others competent to criticise them.

(2) There are nine chapters for which there is at present no subject matter. As many as possible of these have been referred to referees or collaborators asking for recommendations as to material available for insertion.

(3) Twenty-six chapters have been returned by reviewers, their comments and suggestions noted, revisions made in accordance therewith, where approved by the Committee, and unsettled questions have been taken up by further correspondence and inquiry.

(4) Eight chapters were still in the hands of reviewers with the prospect that they would be returned by the time of the meeting in November. This includes the appendix which Mr. Haskins has kindly consented to revise for the Committee.

As to general editorial policy the Committee has not departed greatly from that of the previous edition. General definitions of terms and explanatory notes as given in the 1930 edition, p. XVII, have been followed. Saving of space to offset added new material seemed advisable and the Committee has sought to do this wherever such saving could be effected without sacrifice of clarity. Chiefly to this end has been the omission of descriptions of reagents and/or apparatus in the introduction of sections wherever such can be identified or described clearly by parenthetical statements in the text of methods.

The matter of suitable and convenient alcohol tables has been a debated topic in the last three revisions at least. With increasing attention



being given to alcoholic liquors and beverages it is important that some common ground be found and tables adopted that will be most useful to the majority of workers in the field of beverage, liquor, and drug control. Special attention has been given to this feature by a sub-committee and the following plan suggested for the present revisions:

- (1) Deletion of present Tables 19, 20 and 21 (6 pages).
- (2) Substitution of a new table (a) based upon apparent specific gravities (sp. gr. in air); (b) range of alcohol from 0 to 100%; (c) arrangement according to definite increments in specific gravity instead of increments in proportion of alcohol; (d) alcohol expressed in per cent by volume for a series of temperatures within the range of usual working conditions.
- (3) Insertion of a conversion table for the conversion of per cent alcohol by volume to per cent by weight.

This will require more space than the present plan but if the size of type used in the third edition is agreed upon as satisfactory, the extra pagination will not exceed 9 pages.

The Committee has endeavored to amplify the selected references at the end of each chapter so that a more complete picture of the development of methods will be afforded. It often happens that references now cite only committee recommendations and affirmative actions. The reports and data upon which methods are based frequently are not cited. To make the references complete in this respect is too much of a task to be accomplished at the time of any one revision. It is probably an editorial matter but an interim undertaking in which chapter referees can help, perhaps by the appointment of special associate referees on references.

At the close of the meeting the Committee will give attention to the changes adopted at this session and revise the various chapters accordingly.

E. M. BAILEY

Approved.

#### REPORT OF EDITORIAL COMMITTEE OF PRINCIPLES AND PRACTICE OF AGRICULTURAL ANALYSIS

Since the last meeting the Association has sold five copies of the new edition of Volume II of Wiley's "Principles and Practice of Agricultural Analysis." This brings the total sales by the Association to 60 copies; 16 copies had previously been given to authors and reviewers, which leaves 24 copies still unsold of the original 100 that were purchased from the Chemical Publishing Company.

As stated in last year's report the very unsatisfactory reception attending the sales of this volume has led to the conclusion that it would be highly undesirable for the Association to continue further work upon the preparation of Volume III of the "Principles and Practice," which

was to deal with the analysis of agricultural products. It was recommended, therefore, at last year's meeting that the original plan of publishing this volume be abandoned and that the manuscripts thus far submitted be returned to the authors with a letter of regret and a copy of the report. The Executive Committee later advised that some of these chapters might be published as separates. It is hoped that some such plan may be worked out, but it is doubtful if the publication and sale of analytical monographs can be accomplished successfully by the Association. More satisfactory arrangements with a better prospect of royalties can probably be secured by personal contact between authors and publishers. In making this final report the Editorial Committee of Wiley's "Principles and Practice of Agricultural Analysis" would recommend that its labors be discontinued and the committee discharged.

C. A. BROWNE

Approved.

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No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

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## REPORT OF COMMITTEE ON DEFINITION OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

### *Final Adoption as Official*

#### 1. MANGANESE

The water-soluble or available manganese in fertilizers shall be expressed as manganese (Mn).

#### 2. MANGANESE SULFATE

The term *manganese sulfate*, when connoting an ingredient of a mixed fertilizer, shall designate anhydrous manganous sulfate ( $\text{MnSO}_4$ ).

#### 3. CYANAMID

*Cyanamid* is a commercial product composed chiefly of calcium cyanamid ( $\text{CaCN}_2$ ), and it shall contain not less than twenty-one per cent (21 %) of nitrogen.

#### 4. HIGH-CALCIC PRODUCTS

*High-calcic products* are materials in which 90 per cent or more of the total calcium and magnesium oxide content consists of calcium oxide.

#### 5. HIGH-MAGNESIC PRODUCTS

*High-magnesian products* are materials in which more than 10 per cent of the total calcium and magnesium oxide content consists of magnesium oxide.

### *Previous Final Adoption as Official*

Requiring unanimous consent to delete the words now appearing in brackets, making the reading:

*Interpretation of the word "lime" as applied to fertilizers:* The term "lime" shall not be used in the registration, labeling, or guaranteeing of fertilizers or fertilizer materials unless the lime is in a form, or forms, to neutralize soil acidity.

*Quick lime, burned lime, caustic lime, lump lime, unslaked lime:* Change the present wording to read "These designations shall apply to calcined materials, the major part of which is calcium oxide, in natural association with a lesser amount of magnesium oxide, and which is capable of slaking with water."

*Hydrated or slaked lime:* This is a dry product consisting chiefly of the hydroxide of calcium and oxide-hydroxide of magnesium.

*Agricultural liming material:* Such a material shall be one whose calcium and magnesium content is capable of neutralizing soil acidity.

### *Second Reading as Tentative*

#### 1. AIR-SLAKED LIME

*Air-slaked lime* is a mixture of  $\text{CaO}$ ,  $\text{Ca(OH)}_2$ , and  $\text{CaCO}_3$  in variant proportions, and it results from exposure of quick lime.

#### 2. PULVERIZED LIMESTONE

*Pulverized limestone* is the product obtained by grinding either calcareous or dolomitic limestone so that all of the material will pass a 20-mesh sieve and at least seventy-five (75%) per cent shall pass a 100-mesh sieve.

#### 3. GROUND LIMESTONE

*Ground limestone* is the product obtained by grinding either calcareous or dolomitic limestone so that all of the material will pass a 10-mesh sieve and at least fifty per cent (50%) shall pass a 100-mesh sieve.

#### 4. GROUND SHELLS

*Ground shells* is the product obtained by grinding the shells of mollusks so that not less than fifty per cent (50%) will pass a 100-mesh sieve. The product shall also carry the name of the mollusk from which said product is made.

#### 5. GROUND SHELL MARL

*Ground shell marl* is the product obtained by grinding natural deposits of shell marl so that at least seventy-five per cent (75%) will pass a 100-mesh sieve.

#### 6. SUPERPHOSPHATE

*Superphosphate* is a commercial product, the phosphoric acid content of which is due chiefly to mono-calcium phosphate.

#### 7. ACID-FORMING FERTILIZER

*An Acid-Forming Fertilizer* is one that is capable of increasing the residual acidity of the soil.

#### 8. NON-ACID FORMING FERTILIZER

*A Non-Acid Forming Fertilizer* is one that is not capable of increasing the residual acidity of the soil.

### *First Reading as Tentative*

#### 1. DOLOMITE

*Dolomite* is a mineral composed chiefly of carbonates of calcium and magnesium in practically molar proportions.

#### 2. AMMONIATED SUPERPHOSPHATE

*Ammoniated superphosphate* is the product obtained when superphosphate is treated with ammonia either alone or with other forms of dissolved nitrogen.

#### 3. PRIMARY FERTILIZER COMPONENTS

*Primary Fertilizer Components* are those at present generally recognized by law as necessary to be guaranteed in fertilizers; namely, nitrogen, phosphoric acid, and potash.

#### 4. SECONDARY FERTILIZER COMPONENTS

*Secondary Fertilizer Components* are those other than the "primary fertilizer components" and which are essential to the proper growth of plants and may be

needed by some soils. Some of these components are sulfur, manganese, copper, zinc, boron, magnesium, and calcium.

#### 5. BAT MANURE

*Bat manure* is the dry excrement of bats.

#### 6. BAT GUANO

*Bat guano* is partially decomposed bat manure.

W. H. MACINTIRE, *Acting Chairman*

H. D. HASKINS

G. S. FRAPS

L. S. WALKER

L. E. BOPST

Approved.

### REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

No report of this Committee can be made without expressing a deep appreciation of the hours of work spent by referees, associate referees, and collaborators to the end that each annual meeting will be a success in the accomplishment of the purposes of the Association. The Chairman also wishes at this time to thank the members of the Committee for the thoroughly effective manner in which the work of Subcommittees A, B, and C has been done.

The enlargement of the scope of our work has continued. The program has been supported this year by reports in the new fields of microbiological and vitamin methods, and in the extended fields of the alcoholic beverages section. Refereeships on carotin in feeding stuffs and on disinfectants have been added. As a result of these enlarged activities and the splendid referee response to them, the problem of this Committee to accomplish its assignment has necessarily been made harder. Members of the subcommittees find it necessary to spend practically all of their time at the meeting in committee rooms. In an effort to alleviate this congestion it is recommended that the membership of this Committee be increased by three additional members, permitting the formation of a new subcommittee, D, and a reassignment of subjects.<sup>1</sup>

In connection with the revision of *Methods of Analysis*, it is recommended that the changes that have been, or will be made, be referred to referees for clarification of procedures, and that those that are editorial in character be approved by the Revision Committee and concurred in by this Committee without final presentation in detail.<sup>2</sup>

H. A. LEPPER

Approved.

<sup>1</sup> This action was supported by the adoption of an amendment to the Constitution.

<sup>2</sup> This recommendation was approved by vote of the Association.

## REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES

By H. R. KRAYBILL (Purdue University Agricultural Experiment  
Station, Lafayette, Ind.), *Chairman*; G. L. BIDWELL and  
G. E. GRATTAN

The following recommendations submitted by Subcommittee A were approved by the Association unless it is otherwise stated, and unless given otherwise all references are to *Methods of Analysis, A.O.A.C.* 1930.

### STANDARD SOLUTIONS

It is recommended—

- (1) That the methods submitted by the referee for the preparation and standardization of acid and alkali be adopted as tentative methods (see p. 107).
- (2) That the referee attempt to devise a direct method for standardizing acids.
- (3) That methods be studied for the preparation of standard solutions other than those used in acidimetry and alkalimetry.

### INSECTICIDES, FUNGICIDES AND CAUSTIC POISONS

It is recommended—

- (1) That the methods for the determination of sodium hypochlorite, available chlorine, chloride chlorine, sodium hydroxide, and carbon dioxide, *This Journal*, 18, 63, (1935), be adopted as official (final action).
- (2) That the method for the determination of available chlorine in calcium hypochlorite and bleaching powder, *This Journal*, 18, 64 (1935), be adopted as official (final action).
- (3) That the methods for the determination of active chlorine, total chlorine, and sodium in chloramine-T, *This Journal*, 18, 66, (1935), be adopted as official (final action).
- (4) That the methods for the analysis of London purple (p. 42, 32–41) be deleted. London purple has largely disappeared from the market, and at present there is very little need for these methods.
- (5) That the volatilization method for the determination of fluorine in insecticides and fungicides (p. 42, 146–149) be deleted. The method is long and tedious and does not give accurate results.
- (6) That to the hydrogen peroxide method for the determination of formaldehyde (p. 56, 130) the statement suggested by the referee be added (see p. 70).
- (7) That methods of analysis for derris and cubé be studied.

### SOILS AND LIMING MATERIALS

It is recommended—

- (1) That studies of methods for the determination of the hydrogen-ion concentration of acid and alkaline soils be continued.
- (2) That studies on liming materials be continued.

(3) That further study be conducted to perfect a procedure for the recovery of fluorine from plant ash.

(4) That study be made of the maximum quantity of organic matter permissible in the determination of fluorine and that a method covering these conditions be studied collaboratively.

(5) That the fusion and combustion methods submitted by the associate referee for the determination of iodine in soils be adopted as tentative (see p. 66), and that further collaborative work be done on these methods.

(6) That the method for the determination of arsenic in soils, which was adopted as tentative last year, be further studied.

#### FEEDING STUFFS

It is recommended—

(1) That an associate referee be appointed to work on fluorine in feeds in cooperation with the Associate Referee on Fluorine in Insecticides.

(2) That a study be made of a method for the detection of adulteration of cod liver oil.

(3) That a study be made of a method for the detection of adulteration of condensed and semi-solid milk products.

(4) That a method for the detection of ergot in rye middlings, rye screenings, and distillers rye dried grains be studied.

(5) That further study be given to the microanalytical detection of iodine in feeding stuffs.

(6) That a study be made of the detection of mineral adulterants in feeds.

(7) That the method proposed by Elmslie and Caldwell be further studied and that other simplified procedures for the determination of iodine in mineral mixed feeds be considered for collaborative work.

(8) That the revision submitted by the associate referee (see p. 93) be substituted for the present tentative method for the determination of calcium oxide in mineral feeds (p. 287, 36).

(9) That a committee be appointed to study the whole moisture question with the object of selecting one official vacuum method for all materials and correlating the other moisture methods as much as possible.

(10) That the chairman of the Committee on Moisture be the Associate Referee on Moisture.

(11) That study be continued on the present tentative method for Vitamin D assay by preventive biological test with a view to possible revision and collaborative work.

(12) That the investigational work on the antirachitic potency of proprietary feeds be continued.

(13) That the modified alkaline titration method using  $\text{NH}_3$  and  $\text{KI}$ , described by the associate referee, be adopted as a tentative method (see p. 94).

(14) That further study be given to the Prussian blue method and the colorimetric method of Francis and Connell, *J. Am. Chem. Soc.*, **35**, 1624 (1913).

(15) That the qualitative test described by the associate referee for the detection of cyanogenetic glucosides be adopted as tentative (see p. 94).

(16) That the use of a photoelectric colorimeter for the determination of hydrocyanic acid in glucoside-bearing materials be further studied.

(17) That work on the modified hydrolysis method in connection with dairy products be discontinued.

(18) That studies on the proposed method for the determination of Vitamin B (B<sub>1</sub>) or the antineuritic vitamin in feeding stuffs be continued.

(19) That collaborative work on this subject be continued and extended.

(20) That the method for the determination of salt (p. 278, 9) be adopted as official (final action).

(21) That the study of various factors affecting bone ash determinations be continued.

(22) That the study of dissection and method of preparation of the bone sample for analysis be undertaken.

(23) That extraction methods using different lipoid solvents be further critically evaluated.

(24) That study of the mechanical classification of alfalfa products be discontinued.

(25) That the qualitative tests for proteins described last year, *This Journal*, **18**, 81 (1935), be adopted as official (final action).

(26) That study of qualitative tests for proteins be continued.

#### SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the table of conversion factors for different saccharimeter scales, recommended last year, *This Journal*, **18**, 162 (1935), be published in the next edition of *Methods of Analysis*. These factors, like the values for atomic weights, are of course subject to such revision as future re-determinations of these constants may determine.

(2) That the study of the effect of clarifying agents upon the polarization of food products be extended to the examination of jellies and other pectin-containing materials.

(3) That the action deleting the method for the determination of invert sugar in honey taken in 1931, *This Journal*, **15**, 78 (1932), be rescinded, and that the method then adopted be deleted, pending further study.

(4) That studies on the determination of moisture in honey be continued.

(5) That work on maple flavor concentrates and imitations be continued.

(6) That the modification of the official procedure for the determination of Canadian lead value suggested by C. H. Jones, *viz.*, the addition of asbestos to the precipitate before filtration be subjected to collaborative study.

(7) That the refractive indices of invert sugar solutions be determined.

(8) That the change in refractive indices with change of temperature be determined for such products as invert sugar solutions, table sirups, etc.

(9) That study on polariscopic methods be continued along the lines covered by the recommendations made and approved in 1931 and 1932 and continued in 1933.

(10) That study of chemical methods for reducing sugars be continued.

(11) That the subject of lead precipitate be further studied.

#### FERTILIZERS

It is recommended—

(1) That the work formerly assigned to the Associate Referee on High Analysis Fertilizers be divided between two associate referees, one to be the Associate Referee on Magnesia and Manganese, and the other to be the Associate Referee on Acid-forming and Base-forming Quality of Fertilizers.

(2) That sec. 14(a), p. 18, line 3, be changed as recommended by the referee (see p. 68).

(3) That a further study be made of the application of filter pulp in the Shimer filter for the filtration of citrate-insoluble residues

(4) That a collaborative study be made of methods for determining the availability of calcined phosphate.

(5) That the method adopted last year as tentative for the determination of water-insoluble nitrogen in cyanamid be adopted as official (first action).

(6) That sec. 32(b), p. 22, be changed as recommended by the referee (first action). (See p. 68.)

(7) That the additions recommended by the referee be made to the nitrogen activity methods, 36, 37, 39, pp. 23 and 24 (see p. 68).

(8) That the modified Pierre method for determining whether a fertilizer is acid-forming or non-acid-forming, *This Journal*, 18, 236 (1935), be adopted as tentative.

(9) That in the Pierre method the elimination of water-soluble material coarser than 20-mesh by wet sieving, before applying the method, be studied next year.

(10) That the basicity of phosphate rock and other factors that affect the method be studied further.



(11) That the Hoffman method submitted by the referee for the determination of total magnesium be adopted as tentative (see p. 00).

(12) That further collaborative work on this subject be carried out next year.

(13) That the study of methods for the determination of available magnesium be continued.

(14) That secs. 42(a) and (b); 43(a) and (b), pp. 25 and 26, Mixed Fertilizers, be changed as recommended by the associate referee (final action).

(15) That further study be given to the following suggested change in the last two sentences of sec. 43(a), p. 26: "Weigh and remove the chloroplatinate precipitate by washing with hot water, using slight suction. Wash with 80% alcohol three times, dry as before, and weigh. Loss =  $K_2PtCl_6$ . Calculate to  $K_2O$ ."

(16) That studies be made of the errors resulting from the non-uniformity of the 2.5 g. samples weighed out for the potash determination.

#### PLANTS

It is recommended—

(1) That secs. 1, "Directions for Sampling"; 6, "Iron and Aluminum"; and 7, "Micro Method for Iron Only," be made official (first action).

(2) That secs. 10, "Micro Method, Calcium," and 28, "Method II, Micro Method Phosphorus," be made official (final action).

(3) That sec. 9, part 1, official method for calcium be deleted (final action under suspension of the rules).

(4) That sec. 13, official method for the determination of magnesium, be changed as suggested by the referee (first action, see p. 71).

(5) That the titanous chloride titration method for the determination of iron given in the report of the referee be further studied collaboratively.

(6) That the following methods given in the report of the referee be made tentative and that they be studied collaboratively: perchloric acid method for potassium and the magnesium uranyl acetate method for sodium (see p. 71).

(7) That the method for the determination of fluorine given in the report of the referee be compared with other methods and studied collaboratively.

(8) That the studies on different forms of nitrogen be continued.

(9) That further collaborative study be made of the combustion method for the determination of iodine in plant material, adopted as a tentative method last year, *This Journal*, 18, 73 (1935).

(10) That the method submitted by the associate referee for the determination of the chlorine in plant material be adopted as a tentative method (see p. 72).

(11) That the chlorine method be studied further with reference to

the kinds of material suitable for treatment by the method and details of preparation and use of the aqueous iodine solution.

(12) That the tentative volumetric permanganate method for the determination of reduced copper, (p. 113, 43(a) and (b)), be replaced by the procedure submitted by the associate referee (see p. 72).

(13) That the methods for the determination of sucrose in plants be further studied.

(14) That studies be made of methods for the determination of starch in plants.

(15) That the method for the determination of lead referred to by the referee be compared with other methods and studied collaboratively.

#### LIGNIN

It is recommended that the modified fuming hydrochloric acid method submitted by the referee, *This Journal*, 15, 126 (1932), be adopted as a tentative method for the quantitative estimation of lignin.

#### ENZYMES

It is recommended—

(1) That the studies on catalase be continued.

(2) That the method for the assay of papain be further studied, preferably on more active preparations, including those of bromelin, and that further information regarding the best method of activating the enzyme be sought.

#### PAPER AND PAPER MATERIALS

It is recommended that no methods on paper and paper materials be adopted by this Association and that the subject be dropped from *Methods of Analysis*.

#### WATERS, BRINE, AND SALT

It is recommended—

(1) That the method for the determination of fluorine in water described by the referee be adopted as a tentative method (see p. 99).

(2) That Method I, official for the determination of arsenic (p. 420, 78 and 79) requiring the use of the Marsh-Berzelius apparatus, be dropped (final action under suspension of the rules). This method has become obsolete through the use of the Gutzeit method.

(3) That study of the determination of small quantities of iodine in iodized salt be continued.

(4) That further study be made of the methods described by the Associate Referee on Mineral and Effervescent Salts.

#### PAINTS, PAINT MATERIALS, AND VARNISHES

It is recommended—

(1) That the methods for paints, IX, as revised for publication in the 1935 edition of *Methods of Analysis*, be made official (final action).

(2) That the methods for oleoresinous varnishes, IX, as revised for

publication in the 1935 edition of *Methods of Analysis*, be made official (final action).

(3) That the methods for raw and boiled linseed oil, *This Journal*, 18, 69 (1935), be adopted as official (first action).

(4) That the heading of the chapter, Paints, Paint Materials, and Varnishes, be revised to read as follows: Paints, Varnishes, and Constituent Materials.

(5) That study on the accelerated testing of paints be continued.

#### VITAMINS

It is recommended—

(1) That a standard cod liver oil for Vitamin D assays be made available.

(2) That further investigation of the use of the vitameter for the determination of Vitamin A be made.

(3) That until sufficient data have been obtained to permit the formulation of a tentative method, the U.S.P. X, 1934, method be used for the sake of uniformity.

(4) That further studies be continued on methods for the determination of vitamin D in milk.

#### LEATHERS AND TANNING MATERIALS

It is recommended—

(1) That the editorial changes suggested by the referee to provide greater conformity with the methods of the American Leather Chemists Association be made in the Chapters on Leathers and Tanning Materials.

(2) That the method for extraction of woods, barks, and spent materials, p. 100, 25(a), be changed as suggested by the referee.

#### DISINFECTANTS

It is recommended that the U. S. Food and Drug Administration method (F.D.A. method) for determining the phenol coefficient (including both *Eberthella typhi* and *Staphylococcus Aureus*), as published in U. S. Dept. Agriculture Cir. No. 198, Dec. 1931, be adopted as a tentative method of this Association.

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### REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES

By A. E. PAUL (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman*; L. B. BROUGHTON and H. J. FISHER

The following recommendations submitted by Subcommittee B were approved by the Association unless it is otherwise stated, and unless given otherwise all references are to *Methods of Analysis*, A.O.A.C., 1930.

## NAVAL STORES

It is recommended—

(1) That in the method for determining petroleum ether-insoluble matter, *This Journal*, 18, 67 (1935), the time of drying the crucible be reduced from 5 to 1.5 hours.

(2) That for the determination of ash, *This Journal*, 18, 67 (1935), the quantity of sample used be 10 grams instead of 5.

(3) That the study of the method for the determination of saponification number be continued.

(4) That the effect of substituting special denatured alcohol No. 30 for 95 per cent alcohol in the determination of acid and saponification number be studied.

## RADIOACTIVITY OF FOODS AND DRUGS

It is recommended—

(1) That the gamma ray method for the determination of radioactivity be adopted as tentative (see p. 101).

(2) That the radon method, now official (first action), be made official (final action).

(3) That the title of the chapter "Radioactivity of Foods and Drugs" (official, first action), be changed to read "Radioactivity" (official final action).

(4) That the emanation method be modified to provide for the presence or absence of appreciable quantities of barium sulfate.

## DRUGS

## MICROCHEMICAL METHODS FOR ALKALOIDS

It is recommended—

(1) That the associate referee's proposed methods for the identification of theobromine and theophylline be adopted as tentative methods (see p. 102).

(2) That this subject be continued and that during the coming year attention be given to apomorphine, hydrastine, pelletierine, and anabasine.

## MICROCHEMICAL METHODS FOR SYNTHETICS

It is recommended—

(1) That the methods submitted by the associate referee for the identification of acetanilid, acetphenetidin, and neocinchophen be adopted as tentative (see p. 103).

(2) That study of this topic be continued, and attention given to barbital, phenobarbital, amytal, and ethylhydrocupreine (optochin).

## HYPOPHOSPHITES

It is recommended that this topic be continued.

**SANTONIN**

It is recommended that the present tentative method for the determination of santonin in santonica be deleted and that the associate referee's proposed method be tentatively adopted (see p. 104).

**BENZYL COMPOUNDS**

It is recommended that this subject be continued.

**RHUBARB AND RHAPONTICUM**

It is recommended that this subject be continued.

**HEXYLRESORCINOL**

It is recommended that this subject be continued.

**ERGOT ALKALOIDS**

It is recommended that this topic be continued.

**GUAIACOL**

It is recommended that this topic be continued.

**BIOLOGICAL TESTING**

It is recommended that this subject be continued.

**IODINE OINTMENT**

It is recommended that study be made of methods for determining separately, iodine in the three forms: free, inorganically combined, and organically combined. Since U.S.P. XI includes a method for the determination of total iodine, it is unnecessary that it be adopted also by this Association.

**ACETPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN**

It is recommended that this topic be continued until methods for the complete separation of the three constituents have been devised and studied.

**PYRIDIUM**

It is recommended that this topic be continued for another year.

**GUMS**

It is recommended that this topic be continued.

**OIL OF PEPPERMINT**

As there is a method for the determination of menthol in the new U.S.P. XI, it is recommended that no further work be done on this subject at the present time.

**PSYLLIUM**

It is recommended that the associate referee's method be tentatively adopted (see p. 104) and that the subject be closed.

**DINITROPHENOL**

It is recommended that this topic be continued.

**THEOBROMINE CALCIUM**

It is recommended—

(1) That the method which was collaboratively studied by the associate referee be adopted as a tentative method (see p. 105).

(2) That the applicability of the method to the assay of tablets be studied.

**CHLORBUTANOL**

It is recommended that this subject be continued.

**ASPIRIN AND PHENOLPHTHALEIN MIXTURES**

It is recommended that this subject be further studied.

**STABILITY OF POTASSIUM IODATE VOLUMETRIC SOLUTIONS**

It is recommended that this subject be continued for one additional year.

**CINCHOPHEN—SODIUM BICARBONATE MIXTURES**

It is recommended that a study of an assay method for cinchophen tablets containing sodium bicarbonate be undertaken.

**PEPSIN IN LIQUIDS**

It is recommended that the edestin method for the determination of pepsin in liquids be studied by the Referee on Enzymes.

**HOMATROPINE IN TABLETS**

It is recommended that a study of this subject be undertaken.

**CHANGES IN METHODS OF ANALYSIS, 1930**

It is recommended—

(1) That the following methods, which are official (first action), be made official (final action): Ephedra, 43; camphor, 55; pyramidon, 98; mercurous chloride in tablets, 128; and cat-eye method for the assay of mydriatics and myotics, 150.

(2) That the melting point method for the determination of acetylsalicylic acid be deleted, and the directions submitted by the referee be substituted (see p. 105).

(3) That the method for the determination of arsenic in iron-arsenic tablets (p. 477, 117) be made official (final action under suspension of the rules).

(4) That the method for the determination of arsenic in iron methylarsenates (p. 478, 120) be made official (final action).

(5) That the method entitled "Arsenic in Arsphenamine and Neoarsphenamine" (p. 479, 121 and 122) be deleted.

(6) That the information regarding the solvent, p. 484, 138(b), be changed to read as follows: "Mix 20 cc. of ether and 80 cc. of chloroform."

(7) That the official method for barbital and phenobarbital be amended to provide for the presence of stearic acid (see p. 106).

(8) That the methods under the subject of chaulmoogra oil (p. 484, 142-145) be deleted because they will be incorporated in U.S.P. XI.

(9) That the method for the determination of dextrose in ampules, *This Journal*, 15, 454 (1932), be deleted in order not to duplicate the method in National Formulary VI.

(10) That 76(b), p. 464, Diacetylmorphine (Heroin) in Tablets, be deleted since it is a duplication.

(11) That since the tests on ephedrine (p. 456) will be incorporated in U.S.P. XI, par. 48 be deleted.

(12) That the present tentative method for mercuric iodide (p. 461, 66) be deleted, since National Formulary VI will include a method for this determination.

(13) That the present tentative ricin method for the determination of pepsin in liquids (p. 464, 78) be deleted.

(14) That since a method for plain phenolphthalein tablets will appear in National Formulary VI, the official iodine method (p. 466, 81-83) be deleted and that the reagents under 81 be used under 86, "Phenolphthalein in Chocolate Products."

(15) That certain descriptive additions and minor changes be made in the present methods for procaine hydrochloride (p. 470) and in the title (see p. 00), and that sections 100(d), (e), and (f) be deleted in order to avoid duplication in U. S. Pharmacopoeia XI.

(16) That the directions for the preparation of bromocresol purple indicator solution submitted by the referee be substituted for methyl red in sections 11 and 106 (see p. 105).

(17) That the sentence in 17(a), line 5, p. 444, "The diluting and heating process must be repeated until acetic acid can no longer be detected in the vapors", be inserted in the method for separation of acetphenetidin and caffeine after the words, "liquid amounts to 8-10 cc."

(18) That the present official method for the determination of strychnine in tablets and liquids (p. 473, 106) be slightly amended (see p. 106).

#### BEERS, WINES, AND DISTILLED LIQUORS

##### ALCOHOL TABLES

It is recommended that the changes in the alcohol tables proposed by the referee be adopted (see p. 75).

##### BEERS

It is recommended—

(1) That the following changes in methods be made: Color, Tentative (2)—use a  $\frac{1}{2}$  inch instead of a  $\frac{1}{4}$  inch cell; Specific Gravity, Official (3)—determine the specific gravity in air at 20/20°, instead of at 20/4°.

(2) That the following methods be deleted: Extract (Method I), Official (5); Extract of Original Wort, Official (8); Degree of Fermentation, Official (9); Total Acid, Official (10) (final action under suspension of the rules).

(3) That the following methods submitted by the referee be adopted as tentative: Apparent Extract; Real Extract, Extract of Original Wort, Real Degree of Fermentation, Total Acid, Carbonic Acid (Gray-Stone method), Sulfurous Acid, Iodine Reaction, Pasteurization, Chlorides, and Methyl Alcohol (see p. 78).

(4) That Table 2 be modified as indicated in the report of the associate referee.

(5) That the title of the chapter be changed to "Malt Beverages, Extracts, and Sirups and Brewing Materials."

#### WINES

It is recommended—

(1) That the minor changes in several methods suggested by the referee be made (see p. 78).

(2) That the following topics be further studied: Total sulfur in sweet wines, watering of white wines, other fruit materials in wine, detection of pomace wine, and the use of lead acetate in the saponification of esters.

(3) That the method recommended by the referee for the determination of titratable acidity with phenolphthalein as inside indicator be adopted as tentative.

#### CORDIALS AND LIQUEURS

It is recommended—

(1) That the following methods, described in the report of the associate referee, be made tentative: 1. Physical Examination; 2. Specific Gravity; 3. Alcohol; 4. Total Solids; 5. Glycerol; 6. Sucrose; 7. Total Acidity; 8. Characteristic Acids; 9. Tartaric Acid; 10. Citric Acid; 11. Malic Acid; 12. Volatile Esters; 13. Gamma Undecalactone (qualitative); 14. Optical Properties of Hydrazine-gamma-undecalactone; 15. Methyl Alcohol; 16. Ash; 17. Soluble and Insoluble Ash; 18. Alkalinity of Soluble Ash; 19. Alkalinity of Insoluble Ash; 20. Phosphoric Acid; 21. Benzaldehyde; 22. Caramel; 23. Coal Tar Colors; 24. Aldehydes; 25. Furfural; 26. Fusel Oil (see p. 78).

(2) That the following new methods be studied: Determination of Volatile Esters, Identification of Gamma-undecalactone, and Benzaldehyde.

#### WHISKEY, RUM AND BRANDY

It is recommended that—

(1) That the paraldehyde test for caramel (p. 148, 80) be deleted, and the zinc acetate method developed by Valaer and Mallory, *This Journal*, 18, 75 (1935), be made official (first action).



(2) That the method for fusel oil be revised to provide for the use of universal standard tapered ground-glass joint connections on the reflux condensers and flasks where the oxidation takes place, and the same kind of connections in the distillation of the valeric acid.

(3) That the new table contained in the report of the associate referee be substituted for Table 76, XVII, p. 147.

#### FUSEL OIL

It is recommended that no change be made in the present fusel oil determination.

#### METHYL ALCOHOL

It is recommended—

(1) That the method for the detection and determination of methyl alcohol in the presence of ethyl alcohol, *This Journal*, 18, 477 (1935), be adopted as tentative after modification in the following particulars: (a) To direct that the aliquot of sample used contain not more than 0.160 gram of methyl alcohol; (b) that 2.5 cc. of 95% alcohol be introduced into the reaction flask immediately before the sample is introduced; (c) that as an advisory measure in all cases 25 cc. of wash solution be used in the receiving flask instead of 25 cc. of absolute alcohol.

(2) That the modified method referred to above be further studied.

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### REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES

By W. B. WHITE (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*; J. O. CLARK and G. G. FRARY

The following recommendations submitted by Subcommittee C were approved by the Association unless it is otherwise stated, and unless given otherwise all references are to *Methods of Analysis*, A.O.A.C., 1930.

#### EGGS AND EGG PRODUCTS

It is recommended—

(1) That the title of section 14, chapter XXIII, p. 248, be changed from "Acidity of Fat" to "Acidity of Ether Extract."

(2) That the rapid method for acidity of ether extract, *This Journal*, 15, 341 (1932), be further studied.

(3) That the method submitted by the associate referee, *This Journal*, 6, 7 (1922), for the determination of ammonia nitrogen be adopted as tentative for liquid eggs and study continued.

(4) That studies of methods for the determination of acid-soluble phosphoric acid be continued.

(5) That study of the official method for the determination of water-soluble and crude albumin nitrogen in dried eggs, *This Journal*, 15, 75 (1932), be continued.

(6) That methods for the determination of sugars, added salt, and qualitative and quantitative glycerol be further studied.

(7) That study of methods for the determination of constituents of the unsaponifiable matter and the determination of fat by acid hydrolysis be continued.

#### FOOD PRESERVATIVES

It is recommended—

(1) That the methods for the preparation of sample for the determination of saccharin, *This Journal*, 17, 194 (1934), be studied collaboratively so as to ascertain their applicability to various types of products.

(2) That further studies based on the work of Tortelli and Piazza be made on the qualitative test for saccharin.

#### COLORING MATTERS IN FOODS

It is recommended—

(1) That collaborative studies be made of the methods submitted by the referee for the separation of ponceau SX and ponceau 3R.

(2) That studies be made on the quantitative separation of sunset yellow FCF from the other permitted dyes.

(3) That the necessary changes be made in the chapter on Coloring Matters in Foods to provide for the addition of three additional colors, sunset yellow FCF, ponceau SX, and brilliant blue FCF.

#### FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the method for the determination of inactive malic acid, *This Journal*, 16, 281 (1933), be adopted as tentative, and that it be further studied with a view to adoption as official.

(2) That the minor changes suggested by the referee be made in the tentative method for the determination of tartaric acid (p. 271, 28). These changes do not affect the underlying principles of the method.

(3) That collaborative study be made of the method for the determination of lactic acid described by the referee.

(4) That studies on soluble solids and effects of acids on sugars on drying be continued.

(5) That studies on pectic acid and electrometric titration of acidity be continued.

#### METALS IN FOODS

It is recommended—

(1) That studies on arsenic isolation, ashing, and sample preparation, and the molybdenum blue method of arsenic determination be continued.

(2) That the amendments to the Gutzeit method for the determination of arsenic (p. 306) proposed by the referee be adopted as official (final action under suspension of the rules).

(3) That studies on increasing the range of the Gutzeit method be continued.

(4) That the precautionary note suggested by the referee be added to the tentative bromate method, *This Journal*, 16, 75 (1933); 17, 70 (1934).

(5) That studies be continued on micro methods for the determination of copper and attention given to methods of the dithizone type.

(6) That the work on zinc methods be placed under a separate associate referee.

(7) That studies on the fluorine method be continued.

(8) That the dithizone titrametric method for the determination of mercury, *This Journal*, 18, 640 (1935), be adopted as tentative.

(9) That studies on selenium methods be continued.

(10) That the colorimetric dithizone method submitted by the referee (see p. 130) and the electrolytic method, *This Journal*, 17, 108 (1934); 18, 315 (1935), for the determination of lead be adopted as tentative.

#### DAIRY PRODUCTS

It is recommended—

(1) That the methods for the determination of ash and total chlorides in cheese, *This Journal*, 18, 401 (1935), be adopted as tentative.

(2) That the method for the determination of lactose in milk, p. 216, 12(b), be corrected and clarified as suggested by the referee (see p. 92).

(3) That to the qualitative test for gelatin (p. 223, 26), there be added the clarifying note suggested by the referee (see p. 92) and that the last sentence of 26 be deleted.

(4) That the method for the determination of added water in cream (p. 225, 32) be made official (final action).

(5) That the American Public Health Association method for sediment in milk (A.P.H.A. Standard Methods of Milk Analysis, 1934, 6th ed., pp. 44-46) be adopted as tentative.

(6) That studies on modifications of the Babcock method for the determination of fat in ice cream be discontinued.

(7) That the Hartmann-Hillig method for the determination of citric acid in milk, *This Journal*, 15, 643 (1932), be adopted as tentative and subjected to further study.

(8) That the method for the determination of fat in dried milk, *This Journal*, 15, 75 (1932), be made official (final action), and that the section "Preparation of Solution" be changed to make the procedures alternative.

(9) That the tentative method for the determination of lactic acid in dried milk, *This Journal*, 18, 78 (1935), be dropped.

(10) That the colorimetric method for the determination of lactic acid in dried milk proposed by the associate referee be further studied, together with other methods.

(11) That study be made of methods for detecting neutralizers in dairy products.

(12) That the determination of fat and Reichert-Meissl value of the same in malted milk by the method published in *This Journal*, 18, 455 (1935) be further studied collaboratively.

(13) That study of methods for the determination of casein in malted milk be continued.

(14) That study of the mounting medium in the method for microscopical identification of malted milk be continued.

(15) That the methods for the determination of casein and albumin in milk be studied with a view to the adoption of not more than one method for each protein.

(16) That the official method for the determination of ash and salt in cheese (p. 239, 193) be dropped (final action).

(17) That the official method for the determination of fat in cheese (p. 239, 97) be dropped (final action).

(18) That Method I, official for the determination of moisture in cheese (p. 238, 91), be amended by the insertion after the word "cheese" in the second line of the words "and process cheese" (final action).

(19) That study of the stirrer method for preparation of sample of butter be continued.

(20) That the indirect method for determining fat (p. 236, 79) be studied to determine the best means of removing the last traces of fat.

(21) That studies on methods for the detection of extraneous matter in butter be continued.

#### OILS, FATS, AND WAXES

It is recommended—

(1) That the method submitted by the Referee on Coloring Matters in Foods for the identification of permitted colors be adopted as tentative (see p. 95).

(2) That a collaborative study be undertaken of Kaufmann's thiocyanogen method, *Z. Unters. Lebensm.*, 51, 15 (1926), as applied to fats.

(3) That the specifications for titer thermometer (p. 317, 14) be dropped (final action under suspension of the rules).

(4) That the specifications for titer thermometer submitted by the associate referee (see p. 95) be adopted as official (first action).

(5) That both the Malfatti and the Stout and Schuette procedures submitted by the associate referee for the preparation of aldehyde-free potassium hydroxide solution be substituted for the present official method, p. 321, 21(b) (first action).

(6) That collaborative studies be continued on methods for the determination of acetyl value and hydroxyl number.

(7) That the study of the refractometric method for the determination of oil in oleaginous seeds be continued.

(8) That tentative Method I for the detection of foreign fats containing tristearin in lard (p. 331, 46) be dropped.

(9) That Method II for the same determination (p. 332, 47) be revised as recommended by the associate referee (see p. 97).

#### NUTS AND NUT PRODUCTS

It is recommended that the methods presented by the referee last year, *This Journal*, 18, 419 (1935), be adopted as tentative.

#### CANNED FOODS

It is recommended—

(1) That the tentative method for the determination of total solids in tomato products (p. 398, 13) be changed as suggested by the referee.

(2) That the tentative method for the determination of insoluble solids in tomato products (p. 398, 14) be amended as suggested by the referee.

(3) That the method for the determination of specific gravity in tomato products submitted by the referee be adopted as tentative (see p. 98).

(4) That studies on methods for quality factors and fill of container be continued.

(5) That the methods for micro analysis of tomato pulps, etc. (p. 400, 26-29, inclusive) be made official (final action under suspension of the rules).

#### VINEGARS

It is recommended—

(1) That methods for the determination of total and soluble ash be further studied, with particular attention given to the use of sucrose or other substances for reducing the time of heating, and to the temperature of ashing.

(2) That methods for the determination of phosphoric acid be further studied in connection with the studies on ash.

(3) That the official method for the determination of solids (p. 357, 54) be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

(4) That methods for the detection of caramel be studied.

#### FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the directions for preliminary physical examination submitted by the referee be inserted below the heading on p. 131 (see p. 74).

(2) That the preliminary procedure suggested by the referee for the determination of the characteristic acids (p. 131, 11) be adopted as tentative (see p. 75).

(3) That the method for the determination of malic acid recommended by the referee, *This Journal*, 15, 648 (1932), be adopted as tentative.

(4) That the method submitted by the referee for the determination of benzaldehyde (see p. 75) be adopted as tentative.

(5) That the method submitted by the referee for the detection of gamma-undecalactone, *This Journal*, 16, 420 (1933), be adopted as tentative.

(6) That the note on the applicability of Method I for the determination of essential oil in extracts, *This Journal*, 15, 539 (1932); 16, 75 (1933), and the note on the method of reading the oil column be inserted as directed by the referee (see p. 75).

#### MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the study of methods for the determination of nitrate and nitrite nitrogen in meat and meat products, including meat extracts and curing solutions, be continued.

(2) That further studies be made of methods for the detection of soy bean flour and dried skim milk in meat products.

#### CACAO PRODUCTS

It is recommended—

(1) That methods for the detection of added lecithin be further studied.

(2) That further collaborative work be done on the crude cellulose method for the determination of shell given in the referee's report.

(3) That the tentative micro method for the detection of shell (p. 162, 30) be dropped.

(4) That the tentative method for separation of fat in cacao products (p. 159, 16) be revised as recommended by the referee (see p. 82).

#### GUMS IN FOODS

It is recommended that the tentative method for gums in cheese, *This Journal*, 18, 79 (1935), be further studied in relation to its applicability to foods other than cheese.

#### BAKING POWDERS AND BAKING POWDER CHEMICALS

It is recommended—

(1) That the Heidenhain method for the determination of carbon dioxide (p. 116, 5) be dropped (final action under suspension of the rules).

(2) That the following explanatory note be added to Method II (p. 120, 15): "A method of industrial application, useful when approximate results are desired."

(3) That the Hartmann methods for the determination of tartaric acid and tartaric radical, *This Journal*, 13, 385 (1930), be studied collaboratively.

(4) That Methods I and II for the determination of lead (pp. 124-126, 33-36, inclusive) be dropped (final action under suspension of the rules).

(5) That the method for the determination of fluorine (p. 126, 38-41, inclusive) be dropped (final action under suspension of the rules).

## CEREAL FOODS

It is recommended—

(1) That the following methods from "Cereal Laboratory Methods" be made tentative: Original ash of phosphated and self-rising flour (Gustafson method), and Total carbon dioxide in self-rising flour (p. 116, b), but reference made to *A.O.A.C., Methods of Analysis*, p. 118, 8-10.

(2) That the tentative method for the determination of unsaponifiable residue (p. 170, 28) be dropped.

(3) That the tentative method for the determination of bleaching chlorine (p. 173, 38) be dropped.

(4) That as suggested by the referee, a precautionary note be added to the method for the determination of chlorides in ash as NaCl (p. 181, 64).

(5) That Method I for the determination of fat (p. 178, 53) be dropped.

(6) That Method II for the determination of fat by acid hydrolysis under "Baked Cereal Products" and under "Macaroni Products" (p. 178, 54, and p. 181, 65) be made official (final action).

(7) That the method for the extraction and identification of added color in macaroni products (p. 181, 72) be dropped (final action under suspension of the rules).

(8) That the method for extraction and identification of added color in macaroni products submitted by the Referee on Coloring Matters be made official (first action). (see p. 83.)

(9) That the question of the desirability of methods of analysis of cereal products being used in the brewing industry be referred to the Referee on Alcoholic Beverages.

(10) That the tentative method for the detection of the presence of whole egg or commercial yolk solids, and the method for the determination of egg solids (p. 182, 73 and 74) be dropped.

(11) That the Walters method for the rapid determination of ash in flour be adopted as tentative (see p. 85).

(12) That further studies be made of rapid methods for the determination of ash in flour, macaroni products, and baked products.

(13) That further studies be made on methods for the determination of salt-free ash in macaroni products and baked products.

(14) That studies be made on the various methods for color value of flour.

(15) That the method submitted by the referee for the detection of benzoyl peroxide bleach in flour, *This Journal*, 18, 493 (1935), be made tentative, and that it be further studied with a view to adoption as official.

(16) That the methods for the determination of chlorine bleach published in *This Journal*, 18, 489 (1935) be studied collaboratively.

(17) That the method submitted by the associate referee for the determination of the diastatic value of flour, *This Journal*, 16, 501 (1933); 17, 65, 397 (1934); 18, 76 (1935), be made official (final action).

(18) That studies be discontinued on the modified Rask method for the determination of starch in flour, and that studies be made on the Mannich and Lenz and the Sullivan methods.

(19) That the two methods for the determination of milk solids in bread submitted in amended form by the referee (see p. 86) be adopted as tentative, and that further studies be made on these methods.

(20) That studies on the detection of rye in wheat flour be discontinued.

(21) That further study be made of methods of extraction and colorimetric determination of alkaloids and other constituents of ergot as a means of determining ergot in rye flour.

(22) That further studies be made of methods for the determination of hydrogen-ion concentration in cereal foods.

(23) That further study be made of the tentative method for the determination of the viscosity of acidulated flour suspensions, *This Journal*, 18, 76 (1935).

(24) That the method for the determination of catalase in flour be further studied, and that the method for the determination of protease in flour be studied collaboratively.

(25) That the methods submitted by the associate referee for examination of brewing materials be adopted as tentative (see p. 86) and inserted in the chapter on Beers.

#### MICROCHEMICAL METHODS

It is recommended that the referee subject methods for which there appears to be need to collaborative study

#### MICROBIOLOGICAL METHODS FOR CANNED FOODS

It is recommended that the various studies be continued, and extended to include sugar.

#### FISH AND OTHER MARINE PRODUCTS

It is recommended that studies be continued on methods for which there appears to be need.

#### SPICES AND CONDIMENTS

It is recommended—

(1) That the method for the determination of total sulfur in mustard (p. 351, 16) be dropped (final action under suspension of the rules).

(2) That the method for the assay of ginger submitted by the referee be adopted as tentative (see p. 98).



CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF  
ANALYSIS MADE AT THE FIFTY-FIRST ANNUAL  
MEETING, NOVEMBER 11-13, 1935<sup>1</sup>

## I. SOILS

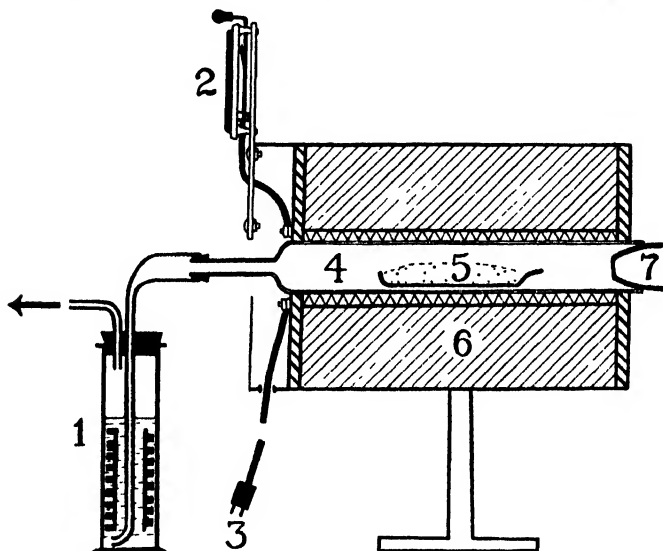
The following fusion and combustion methods submitted by the associate referee for the determination of iodine in soils were adopted as tentative:

## IODINE

*I. Fusion Method.*—Place 5 g of air-dry soil, ground to pass a 100-mesh sieve, 10 g of I-free KOH pellets and 5 ml of H<sub>2</sub>O in a clean 400 ml iron crucible and stir with a clean piece of No. 6 iron wire until most of the pellets have dissolved. Place the crucible in a 4.5 in. Bunsen tripod and heat moderately with the flame of a burner, stirring the contents of the crucible rapidly until the H<sub>2</sub>O has been driven off and a dry granular fused mass is obtained. Avoid heating the crucible to redness after the H<sub>2</sub>O has been expelled. Cool the crucible and add about 50 ml of H<sub>2</sub>O and allow it to stand with occasional stirring until the fused mass has slaked to a sludge. Transfer the contents of the crucible to a 500 ml beaker, police, and wash the inside walls of the crucible thoroughly. Add a small strip of litmus paper, about 0.1 g of K bisulfite, and HCl (1+1), stirring until the contents of the beaker have an acid reaction and a distinct odor of SO<sub>2</sub> can be detected. Add a saturated soln of K<sub>2</sub>CO<sub>3</sub> from a short stem pipet, stirring until the entire mass has an alkaline reaction. Pour the precipitate of silica, Fe and Al hydroxides onto a folded filter and wash thoroughly by the addition of about 25 ml portions of hot H<sub>2</sub>O at a time, allowing each portion to drain through before adding another. (The volume of the filtrate and washings should be approximately 500 ml.) Transfer the filtrate to a porcelain dish and evaporate until a sludge of salts remains. (A small current of compressed air directed on the surface of the soln during heating on the water bath will hasten the evaporation.) Remove the dish from the water bath and add 50 ml of pure 95% ethyl alcohol to the hot sludge of salts. Stir thoroly with a policeman until the dish has attained room temp. (The salts assume a pasty consistency with much stirring while they are hot and this condition facilitates the soln of the KI in the alcohol.) Decant the alcoholic extract through a small folded filter into a beaker, further extract the residue with one 25 and one 10 ml portion of alcohol, and decant through the filter into the beaker containing the first alcoholic extract. Dissolve the salts adhering to the filter paper and those in the dish in hot H<sub>2</sub>O, and evaporate the soln to a sludge. Extract the sludge with one 25 ml and two 10 ml alcoholic portions as directed in the treatment of the first sludge of salts, and repeat the process of extraction, using three 10 ml portions of alcohol. Combine the alcoholic extracts and evaporate to dryness. Dissolve the residue in a small amount of hot H<sub>2</sub>O, rinse into a 250 ml beaker, and evaporate to dryness. Dissolve the residue in a few drops of hot H<sub>2</sub>O, a drop of a saturated soln of K<sub>2</sub>CO<sub>3</sub>, and add 10 ml of alcohol. Stir the precipitated salts rapidly with a glass rod for about 10 min., and decant the alcoholic extract through a small filter into a 150 ml beaker. Further stir the residue, extract with two 5 ml portions of alcohol, decant through the filter into the beaker, rinsing the filter with a few ml of alcohol, and evaporate the extract to dryness. Dissolve the residue, consisting of about 0.05 g, in a few drops of hot H<sub>2</sub>O and rinse into a 25 ml Pt or sillimanite dish, evaporate to dryness, and dry at 100° for 1 hour. Heat the dish at about 400° in an electric

<sup>1</sup> Compiled by Marian E. Lapp, *Associate Referee*. Unless otherwise given all references in this report are to *Methods of Analysis*, A O A C, 1930, and the methods have been edited to conform to the style used in that publication.

furnace having a pyrometer attachment, until the organic matter is burned or charred so that it will not give a turbid soln when  $\text{H}_2\text{O}$  is added. If a turbid soln is obtained, evaporate to dryness and burn again. After cooling the dish, dissolve the residue in a few drops of distilled  $\text{H}_2\text{O}$ , at room temp., filter the soln, and wash into a 30 ml separatory funnel. (The solution should be colorless, slightly alkaline, and have a volume of about 5 ml.) Make slightly acid with a few drops of  $\text{H}_2\text{SO}_4$  (1+1), add about 0.01 g of pure K bisulfite to the separatory funnel, stopper, and shake for a few seconds to reduce any iodate to iodide. Remove the stopper, and add 1 ml of pure  $\text{CS}_2$  and about 2 ml of a 10% soln of pure I-free K or  $\text{NaNO}_2$ . Stopper and shake the separatory vigorously for about 1 min. Place the separatory in a stand and allow the  $\text{CS}_2$  containing the I to collect and settle for about 5 min. If the  $\text{CS}_2$  has a light pink color it contains all the I; if it has a deep pink color, run



Furnace Ready for Use. 1—Gas wash bottle, 2—Rheostat, 3—Power line, 220 volts, 4—Quartz tube, 5—Sample (in sillimanite boat), 6—Electric tube furnace, 7—Stopper (alundum crucible).

it carefully into a centrifuge tube, add 1 ml portions of  $\text{CS}_2$  in the separatory funnel, and repeat the extraction until the last portion has only a slight pink color. Combine the  $\text{CS}_2$  extracts and centrifuge. Place a portion of the clear extract in a micro cup of a colorimeter and compare quickly with a freshly prepared I standard having nearly the same depth of color. Report results in p.p.m.

*II. Volatilization Method by Heating Soil in an Electric Tube Furnace.*—Place in a porcelain boat 25–100 g of soil ground to pass a 2-mm sieve and insert the boat in the silica tube. Connect the first wash bottle with the silica tube by means of a glass thimble made to fit a rubber gasket on the small end of the combustion tube. Connect the wash bottles closely with rubber tubing. Attach the last wash bottle to a suction pump which is regulated to draw the vapors at a moderate rate into the wash bottles during the time the soil is heated. (About 1 hour is required to attain the maximum temp. of the furnace, which is maintained for about 2 hours.) Disconnect the wash bottles, rinse the soln into a porcelain dish, and evaporate to dryness. Dissolve the residue in a few drops of hot  $\text{H}_2\text{O}$ , rinse into a 150 ml beaker, and evaporate until about 2 ml remains. Add to the beaker 10 ml of pure 95% ethyl

alcohol, stir rapidly with a glass rod for about 10 min., and decant the extract through a small filter into a 150 ml beaker. Extract the residue with two 5 ml portions of alcohol and decant through the filter into the beaker. Evaporate the alcohol and wash the residue into a 25 ml Pt or sillimanite dish, evaporate to dryness, dry at 100°, and heat at about 400° for 10 min. in an electric furnace having a pyrometer attachment. Remove the dish from the furnace, cool, and dissolve the residue in a few drops of cold distilled H<sub>2</sub>O. Filter, and wash into a 30 ml separatory. Liberate, absorb, and determine the I as directed in Method I.

## II. FERTILIZERS

(1) In line 3, p. 18, the words "quick-acting filter" were changed to read: "Whatman filter paper No. 5 or other filter paper of equal speed and retentiveness. It is recommended that filtration be made with suction with the use of a Büchner funnel or ordinary glass funnel with a Pt or other cone," and the next sentence, ending with "H<sub>2</sub>O," was followed by the sentence, "If the sample gives a cloudy filtrate, wash with a 5% soln of NH<sub>4</sub>NO<sub>3</sub>" (first action).

(2) The method for the determination of water-soluble nitrogen in cyanamid, adopted as tentative last year, *This Journal*, 18, 62 (1935), was adopted as official (first action).

(3) The following statement was inserted after the first sentence of 32(b), p. 22: "If the fertilizer mixture is greasy or does not wet easily, moisten the dry sample with 7 cc of ethyl alcohol and continue the washing to nearly 200 cc" (first action).

(4) The following additions were made to the nitrogen activity methods, pp. 23, 24: Page 23, 36, line 1—after the words "filter paper" insert the words "wet with alcohol"; 37, line 2—ditto; p. 24, 39, line 2—ditto; line 5, after the words "small beaker" insert the words "wet with alcohol"; 39(b), line 3, after the words "filter paper" insert the words "wet with alcohol."

(5) The modified Pierre method for determining the acid-forming and base-forming quality of fertilizers, *This Journal*, 18, 236 (1935), was adopted as tentative.

(6) The Hoffman method for the determination of total magnesia was adopted as tentative. The method follows:

### TOTAL MAGNESIA

Weigh a 1 g of sample into a 250 cc beaker, cover, add 10 cc of HCl and 30 cc of HNO<sub>3</sub>, and boil gently for 30 min. Remove the beaker from the source of heat, cool, add 6 cc of H<sub>2</sub>SO<sub>4</sub> (1+1), remove the cover, and evaporate until white fumes appear. Cool slightly, wash down the inside surface of the beaker with a jet of H<sub>2</sub>O, and again evaporate until fumes of H<sub>2</sub>SO<sub>4</sub> appear. Cool, add 10 ml of H<sub>2</sub>O, stir thoroughly, and digest on the steam bath for 10-15 min. Remove from the steam bath, add 100 ml of 95% alcohol, stir so that the CaSO<sub>4</sub> is well dispersed throughout the liquid, and allow to stand for 2 hours or longer. Filter by means of suction through a tight plug of filter paper pulp, using a Gooch crucible, and wash 5 times with 5 cc portions of 95% alcohol containing 1 cc of H<sub>2</sub>SO<sub>4</sub> per 100 cc.

Evaporate the alcoholic filtrate as far as possible on the steam bath. Transfer the soln to a 250 cc Erlenmeyer flask, dilute to 75–100 cc, and add 2 g of citric acid and 10 cc of a 25% soln of  $(\text{NH}_4)_2\text{HPO}_4$ . Add  $\text{NH}_4\text{OH}$  until the soln is alkaline to litmus and then add 10 cc in excess. Add 5–10 glass beads, tightly stopper the flask, and shake on a shaking machine for at least 1 hour. Allow to stand in a cool place for 4 hours or preferably overnight. Filter through a tight paper containing a little paper pulp, and wash with  $\text{NH}_4\text{OH}$  (5+95), containing 50 g of  $(\text{NH}_4)_2\text{HPO}_4$  per liter, until the precipitate and paper are free from Fe and Al. Pass 25 ml of hot HCl (5+95) through the paper into the flask, transfer the soln to a 250 cc beaker, and wash the paper and flask thoroughly with more of the diluted acid. To the soln in a volume of 50–75 cc and containing no glass beads, add 0.5 ml of a 25% soln of  $(\text{NH}_4)_2\text{HPO}_4$ , cool, and then add  $\text{NH}_4\text{OH}$  slowly and with stirring until the soln is alkaline to litmus. Stir for a few minutes, then add 3–4 cc of  $\text{NH}_4\text{OH}$  and allow to stand for 4 hours or overnight. Transfer the precipitate to a small filter or filtering crucible and wash with  $\text{NH}_4\text{OH}$  (5+95). Ignite slowly in a crucible at a temp. below  $900^\circ$  until the carbon is burned (preferably in a muffle furnace with pyrometric control), and then at about  $1,100^\circ$  for 1–2 hours. Cool, and weigh.

The residue consists of  $\text{Mg}_2\text{P}_2\text{O}_7$  and possibly  $\text{Mn}_2\text{P}_2\text{O}_7$  and  $\text{Ca}_3(\text{PO}_4)_2$ . If the alcoholic filtrate was clear, the  $\text{Ca}_3(\text{PO}_4)_2$  will not exceed 0.3 mg and can be neglected. The correction for Mn is made as follows: Dissolve the residue in 10 cc of  $\text{H}_2\text{SO}_4$  (1+9), transfer the soln to a 250 cc Erlenmeyer flask, and add 50 cc of  $\text{HNO}_3$  (1+3), 2 cc of sirupy  $\text{H}_3\text{PO}_4$ , sp. gr. 1.7, and 0.2 g of  $\text{KIO}_4$ . Boil for 15–20 min., cool, and dilute to a convenient volume. In another flask containing the same amounts of the reagents treated in a similar way, match the color by adding a standard soln of  $\text{KMnO}_4$ , or compare with a standard soln of  $\text{KMnO}_4$  in a colorimeter. From the volume of the soln of permanganate required, or the reading of the colorimeter, calculate the weight of  $\text{Mn}_2\text{P}_2\text{O}_7$  in the residue. Subtract this weight from the total weight, and regard the difference as  $\text{Mg}_2\text{P}_2\text{O}_7$ , which contains 36.21 % of  $\text{MgO}$ .

(7) With slight editorial changes sections 42(a) and 43(a), pp. 25 and 26, as recommended for adoption (first action) last year, *This Journal*, 18, 63 (1935), were adopted as official (final action).

(8) The changes made in sections 42(b), p. 25, and 43(b) and (c), p. 26, last year, *This Journal*, 18, 63 (1935), were adopted as official (final action).

### III. SEWAGE\*

### IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

### V. AGRICULTURAL DUST\*

### VI. INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

(1) The method for the determination of sodium hypochlorites, available chlorine, chloride chlorine, sodium hydroxide, and carbon dioxide, *This Journal*, 18, 63 (1935), were adopted as official (final action).

(2) The method for the determination of available chlorine in calcium hypochlorite and bleaching powder, *This Journal*, 18, 65 (1935), was adopted as official (final action).

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\* Subjects for future study.

(3) The methods for the determination of active chlorine, total chlorine, and sodium in chloramine-T, *This Journal*, 18, 65 (1935), were adopted as official (final action).

(4) The methods for the analysis of London purple (p. 42, 32-41) were dropped.

(5) The volatilization method for the determination of fluorine (p. 59, 146-49), was dropped.

(6) The following statement was added to the hydrogen peroxide method for the determination of formaldehyde (p. 56, 130):

If the formaldehyde soln contains an appreciable quantity of free acid, titrate a separate portion and calculate the acidity as percentage of formic acid. Make correction for this acidity in calculating the percentage of formaldehyde.

## VII. CAUSTIC POISONS

No additions, deletions, or other changes.

## VIII. NAVAL STORES

(1) In the method for determining petroleum ether-insoluble matter in rosin, *This Journal*, 18, 67 (1935), the time of drying the crucible was reduced from 5 to 1.5 hours.

(2) The quantity of sample to be used in the determination for ash in rosin, *This Journal*, 18, 67 (1935), was changed from 5 to 10 grams.

## IX. PAINTS, PAINT MATERIALS, AND VARNISHES

(1) The methods for paints, as revised for publication in the 1935 edition of *Methods of Analysis*, were adopted as official (final action).

(2) The methods for oleoresinous varnishes, as revised for publication in the 1935 edition of *Methods of Analysis*, were adopted as official (final action).

(3) The methods for raw and boiled linseed oil, *This Journal*, 18, 69 (1935), were adopted as official (first action).

(4) The heading of the chapter on Paints, Paint Materials, and Varnishes, was revised to read, "Paints, Varnishes, and Constituent Materials."

## X. LEATHERS

No additions, deletions, or other changes.

## XI. TANNING MATERIALS

The tentative method for the extraction of woods, barks, and spent materials, p. 100, 25(a), was revised as suggested by the referee.

## XII. PLANTS

(1) The following methods were adopted as official, first action: Directions for Sampling (p. 102, 1); Iron and Aluminum (p. 103, 6); and Micro Method for Iron Only (p. 103, 7).

(2) The micro method for the determination of calcium (p. 105, 10) was adopted as official (final action).

(3) Part one of the official method for the determination of calcium (p. 104, 9) was deleted (final action under suspension of the rules).

(4) The official method for the determination of magnesium (p. 106, 13) was changed (first action) to read as follows:

To the combined filtrates and washings from the Ca determination, 9, add 30 cc of  $\text{HNO}_3$  and evaporate to dryness to decompose the  $\text{NH}_4$  salts. Take up with 5 cc of  $\text{HCl}$  and make to a volume of about 100 cc with  $\text{H}_2\text{O}$ . Add 5 cc of a 10% soln of Na citrate and 10 cc of a 10% soln of  $(\text{NH}_4)_2\text{HPO}_4$  or enough to precipitate all the Mg. Add  $\text{NH}_4\text{OH}$  (1+4), with constant stirring, until the soln is faintly alkaline, then add about 5 cc of  $\text{NH}_4\text{OH}$ , stir vigorously with a rubber policeman until the precipitate becomes granular, and allow to stand in a cool place overnight. Filter, and wash free from chlorides with cold  $\text{NH}_4\text{OH}$  (1+10). Ignite and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . Calculate and report the results as percentage of  $\text{MgO}$  (first action).

(5) The following perchloric acid method for the determination of sodium and potassium was adopted as tentative:

#### SODIUM AND POTASSIUM

##### *Perchloric Acid Method*

Prepare the material according to the directions for the determination of sodium and potassium (p. 106, 15) until the heavy metals have been removed and the two elements are in the form of the chlorides. (Sulfates must be absent.) Add 3-5 cc of 60%  $\text{HClO}_4$ . Evaporate to dryness, dissolve in hot  $\text{H}_2\text{O}$ , and again evaporate to dryness. Heat to  $350^\circ$ . Cool, and weigh if it is desired to obtain the combined perchlorates. Add 10-20 cc of a soln of anhydrous ethyl acetate and C. P. normal butyl alcohol in equal proportions by volume. Digest near the boiling point for several minutes. Decant into a Gooch crucible. Wash once or twice by decantation with a few cc of the acetate-alcohol mixture. Dissolve in the minimum quantity of  $\text{H}_2\text{O}$ , evaporate to dryness, and extract as before. Filter, and wash several times with 1 cc of the acetate-alcohol mixture; dry in an oven at  $110^\circ$  for several minutes and heat at  $350^\circ$  for 15 min. Cool, and weigh. Potassium perchlorate  $\times 0.28218$  = the potassium content. Calculate the sodium by difference.

(6) The following magnesium uranyl acetate method for the determination of sodium was adopted as tentative:

#### SODIUM

##### *Magnesium Uranyl Acetate Method*

#### REAGENT

*Soln A.*—To 85 g of crystallized uranyl acetate and 60 g of glacial acetic acid, add distilled  $\text{H}_2\text{O}$  to make 1000 cc.

*Soln B.*—To 500 g of crystallized magnesium acetate and 60 g of glacial acetic acid, add distilled  $\text{H}_2\text{O}$  to make 1000 cc.

Heat each soln separately to about  $70^\circ$  until all the salts are dissolved. Mix the two solns at this temp. and allow to cool to  $20^\circ$ . Place the vessel containing the mixed reagent in  $\text{H}_2\text{O}$  at  $20^\circ$ , and hold at this temp. for an hour or two until the slight excess of salts is crystallized out. Finally filter the reagent through a dry filter into a dry bottle.

## DETERMINATION

Moisten 1-10 g of the sample with  $\text{H}_2\text{SO}_4$  (1+10), dry in an oven, and ignite in a muffle at a low red heat to destroy the organic matter. Heat the residue on a steam bath with 2-5 cc of  $\text{HCl}$ , add about 40 cc of  $\text{H}_2\text{O}$ , and heat to boiling. Add a sufficient amount of  $\text{CaCl}_2$  soln to precipitate all the phosphates. Precipitate the phosphates by making slightly alkaline with  $\text{NH}_4\text{OH}$ . Filter, and evaporate to 5 cc or less if no salts separate. Cool. Add 100 cc of the magnesium uranyl acetate reagent, place the mixture in a water bath at  $20^\circ$  and either stir vigorously for 45 min. or let stand for 24 hours. Filter with suction and wash with 95% alcohol saturated with sodium magnesium uranyl acetate. Dry at  $105\text{--}110^\circ$  for 30 min., cool, and weigh. Weight of sodium magnesium uranyl acetate  $\times 0.0153$  = the sodium.

(7) The tentative volumetric permanganate method for the determination of reduced copper, p. 113, 43(a) and (b), was deleted.

(8) The following method for the determination of reduced copper was adopted as tentative to replace the above method:

## REDUCED COPPER

*Volumetric Permanganate Method*

Filter and wash the  $\text{Cu}_2\text{O}$  as directed under 41. With the aid of a stirring rod transfer the asbestos mat and the  $\text{Cu}_2\text{O}$  back into the beaker in which the reduction took place. Rinse the inside of the crucible and the lip of the beaker with 10 cc of a soln of 240.9 g of crystalline ferric  $(\text{NH}_4)_2\text{SO}_4$  and 200 cc of  $\text{H}_2\text{SO}_4$  dissolved in  $\text{H}_2\text{O}$  and made up to 1 liter. Cool the diluted  $\text{H}_2\text{SO}_4$  before adding the salt. Receive the rinsings in the beaker containing the  $\text{Cu}_2\text{O}$ . Holding the crucible over the beaker, stir the contents of the beaker thoroughly with the stirring rod until the  $\text{Cu}_2\text{O}$  has gone into soln. Wash the crucible with about 25 cc of hot  $\text{H}_2\text{O}$  ( $80^\circ$ ), receiving the washings in the beaker. Stir the contents of the beaker and then raise the beaker to see if any undissolved particles of  $\text{Cu}_2\text{O}$  are resting on the bottom. If any undissolved particles are present, press out each one with the point of the stirring rod until all have gone into soln. Add about 125 cc more of hot  $\text{H}_2\text{O}$ . Add 1 drop of a soln of 0.15 g of orthophenanthroline monohydrate and 0.07 g of ferrous sulfate in 10 cc of  $\text{H}_2\text{O}$ . Titrate at once with continual stirring with 0.05  $N$   $\text{KMnO}_4$ . (In a long titration it is best to add the indicator just before the end point is reached.) Standardize the  $\text{KMnO}_4$  as follows: Dry overnight about 0.5 g of Na oxalate (U.S. Bureau of Standards) in an oven at  $100^\circ$  and carefully weigh out into beakers 3 samples of about 0.10-0.15 g each. Dissolve each sample in about 100 cc of  $\text{H}_2\text{O}$ , add 5 cc of  $\text{H}_2\text{SO}_4$  (1+1), warm to  $70^\circ$  and titrate the  $\text{KMnO}_4$  against this soln, stirring the liquid vigorously and continuously. Subtract from the titration the excess  $\text{KMnO}_4$  needed to obtain the end point color as determined by matching the color in another beaker containing the same bulk of acid and hot  $\text{H}_2\text{O}$ . The temp. of the soln should not be below  $60^\circ$  by the time the end point is reached. 1 cc of 0.05  $N$   $\text{KMnO}_4$  = 0.00335 g of Na oxalate and 0.0031785 g of Cu, or

$$\text{mg of Cu per cc of KMnO}_4 = \frac{\text{g of Na oxalate} \times 948.8}{\text{cc of KMnO}_4}$$

(9) The following method submitted by the associate referee for the determination of chlorine was adopted as tentative:

## CHLORINE---TENTATIVE

(If bromides or iodides are present in significant quantities the results must be corrected accordingly)

<sup>1</sup> Peters and Van Slyke, Quantitative Clinical Chemistry, vol. 11, p. 845. Williams and Wilkins Co. Baltimore (1932).

## REAGENTS

(a) *Standard potassium iodide soln.*—Weigh out 4.6826 g of the pure, dried salt, dissolve in  $H_2O$ , and dilute to 1 liter. 1 ml  $\approx$  1 mg of Cl.

(b) *Approximately 0.3 N silver nitrate soln.*—Dissolve 48 g of the salt in  $H_2O$ , filter, and dilute to 1 liter. 1 ml  $\approx$  10 mg of Cl (approximately).

(c) *Standard silver nitrate soln.*—Dilute 100 ml of Reagent (b) to about 900 ml and adjust by standardizing against Reagent (a) so that 1 ml  $\approx$  1 mg of Cl.

(d) *Chlorine-free starch indicator.*—For each 100 ml of the final soln take 2.5 g of soluble starch and make to a paste with cold  $H_2O$ . After stirring out the lumps, add 25–50 ml more cold  $H_2O$  and stir or shake for 5 min. Centrifuge, decant, and discard the liquid. Repeat the extraction 3 times and finally transfer the residue to a flask containing the proper quantity of boiling  $H_2O$ . Stir again, allow to come to a boil, cover with a small beaker, and cool under the tap, shaking occasionally.

(e) *Dilute sulfuric acid soln.*—Add 35 ml of  $H_2SO_4$  to 1 liter of  $H_2O$  and cool to room temp.

(f) *Iodine indicator.*—To approximately 20 g of pure I in a 500 ml ground-glass-stoppered bottle add 400 ml of Reagent (e) and shake for 10 min. Decant, and discard the first soln since it may contain iodides. Repeat the process and store this soln in small ground-glass-stoppered bottles.

(g) *Potassium permanganate.*—Dissolve 60 g of the salt in 400 ml of warm  $H_2O$  (about  $50^\circ$ ) and dilute to 1 liter.

(h) *Potassium sulfate—copper sulfate mixture.*—Thoroughly mix 16 parts of  $K_2SO_4$  with 1 part of  $CuSO_4 \cdot 5H_2O$ .

(i) *Wash soln.*—Mix 980 ml of  $H_2O$  with 20 ml of  $HNO_3$ .

## DETERMINATION

Weigh into a beaker such a quantity of the sample as is expected to contain 10–40 mg of Cl. (If more than 4 g is taken, use proportionately more  $HNO_3$  and  $KMnO_4$  soln.) Add 10 ml of the 0.3N  $AgNO_3$  soln and stir until the sample is thoroughly soaked with the soln, adding a little  $H_2O$  or warming if necessary. Add 25 ml of  $HNO_3$ , stir, add 5 ml of the  $KMnO_4$  soln, and stir until the frothing stops.<sup>1</sup> Place the mixture in a water bath or on a hot plate to keep it just below boiling. Stir, and wash down the sides of the beaker at intervals with the least possible quantity of  $H_2O$ . After 20 min, or when there appears to be no further action on the sample, add more of the  $KMnO_4$  soln, a little at a time until the color fades slowly or until twice the amount used at the beginning has been added. Dilute to about 125 ml with boiling  $H_2O$  and heat 10 min. longer. (The beaker may stand in the bath or on the hot plate until ready to filter.) Filter while hot thru Whatman's No. 5, or similar paper, with suction as follows: Place a disk of 30-mesh stainless steel wire gauze or of No. 40 filter cloth in the bottom of a 3 inch Hirsch funnel. Fold a 9 cm paper over the bottom of a No. 11 rubber stopper, shaping it to the funnel by making 9–10 folds up the side of the stopper. Place the paper in the funnel and apply strong suction. Wet the paper and keep it wet while fitting it into the funnel so as to avoid double thicknesses of paper. Thoroughly wash the paper, first with  $H_2O$  then with the wash soln. Discard the washings and rinse out the flask. Pour the supernatant liquid through the filter and transfer the precipitate and sample residue to the filter. If the filtrate is not turbid or only slightly opalescent, wash the precipitate thoroughly, applying the wash soln very gently, but maintaining a strong suction on the filter. If the combined filtrate and washings are clear, test them for Ag. If turbid, re-heat and pass thru the filter, repeating until clear, and finally wash as directed above. If the filtrate does not give a definite test for Ag,

<sup>1</sup> Davies, W. L., *Analyst*, 57, 71–85 (1932).



repeat the determination on a fresh but smaller portion of the sample. Place the filter paper and contents in a Kjeldahl flask and add such quantities of the mixture of  $\text{CuSO}_4$  and  $\text{K}_2\text{SO}_4$  and of  $\text{H}_2\text{SO}_4$  as would be appropriate for a protein determination on the same kind and amount of material and digest in a similar manner. (For 2 g of grass, 8 g of the sulfate mixture and 20 ml of acid are enough.) When the digest is cool, add 75 ml of  $\text{H}_2\text{O}$  and cool to room temp. Titrate the  $\text{Ag}_2\text{SO}_4$  in the Kjeldahl flask with the standard  $\text{KI}$ ,<sup>1</sup> using 5 ml of starch and 30 ml of I as indicator. (The latter is added just before the titration.) Wash down the neck of the flask after each addition of  $\text{KI}$  when near the end point and titrate until the blue color persists after shaking. If less than 30 mg of chlorine is present, add the starch and I at the beginning. If a larger but unknown amount is present, add 2 ml of starch and 10 ml of I at the beginning and titrate until the approach of the end point is seen. Shake vigorously to coagulate the precipitate, add the remainder of the starch and I and proceed to the end point. If a known large amount is present, titrate to within 2 ml of the end point, shake as above, add the indicator reagents, and continue the titration. If the end point is over-run, add 5 ml of the standard  $\text{AgNO}_3$  and titrate again.

Blank determinations are not necessary after the reagents have been tested. If the blanks made by using pure sugar as a sample exceed 0.05 mg, examine the filter paper and the various reagents carefully.

### XIII. FIBERS\*

#### XIV. PAPER AND PAPER MATERIALS\*

As no methods for paper and paper materials are to be adopted by this Association at the present time, this subject was dropped.

#### XV. BAKING POWDERS AND BAKING CHEMICALS

(1) The Heidenhain method for the determination of carbon dioxide (p. 116, 5) was dropped (final action under suspension of the rules).

(2) The following explanatory note was added to Method II (p. 120, 15): "A method of industrial application, useful when approximate results are desired."

(3) Methods I and II, official for the determination of lead (pp. 124-126, 33-37, inclusive), were dropped (final action under suspension of the rules).

(4) The method for the determination of fluorine (p. 126, 38-41, inclusive) was dropped (final action under suspension of the rules).

#### XVI. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

(1) The following directions for preliminary physical examination were inserted below the heading of this chapter (p. 131):

##### PHYSICAL EXAMINATION

Note and record the following: (a) appearance, whether bright or turbid, whether there is any sediment, and whether still or carbonated; (b) color and depth of color; (c) odor, whether fruity, foreign, or artificial; (d) taste, whether tart, sweet, fruity, artificial, or foreign and whether any synthetic substance can be identified by odor or taste.

<sup>1</sup> Christy and Robson, *Biochem. J.*, 22, 571 (1928); Peters and Van Slyke, *loc. cit.*

(2) The following preliminary procedure for the method for the determination of the characteristic acids was adopted as tentative:

#### CHARACTERISTIC ACIDS

##### PRELIMINARY PROCEDURE

(a) *Alcoholic products*.—Proceed as directed under Cordials and Liqueurs (see p. 78).

(b) *Non-alcoholic products*.—Measure out such a volume of sample as contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined, as calculated from the acidity. Evaporate to 30 cc if necessary, add 3 cc of 1 N  $\text{H}_2\text{SO}_4$ , and transfer to a 250 cc volumetric flask, using 10 cc of  $\text{H}_2\text{O}$  and sufficient 95% alcohol to fill the flask to the mark. Mix, and allow to stand 15 min. Filter through a thin layer of absorbent cotton, protecting the liquid against evaporation. Transfer 200 cc of the filtrate to a centrifuge bottle and proceed with the determination of the acid as directed.

(3) The method recommended by the referee for the determination of malic acid, *This Journal*, 15, 648 (1932), was adopted as tentative.

(4) The following method for the determination of benzaldehyde was adopted as tentative:

#### BENZALDEHYDE

Measure into a distilling flask 500 cc of beverage, 100 cc of flavoring sirup, or 10–25 cc of flavor; add 22 cc of alcohol and in the case of the flavor, about 200 cc of  $\text{H}_2\text{O}$  and proceed as directed under Cordials and Liqueurs (see p. 81).

(5) The qualitative test, submitted by the referee for the detection of gamma-undecalactone, *This Journal*, 16, 42 (1933), was adopted as tentative.

(6) The note on the applicability of Method I for the determination of essential oil in extract, *This Journal*, 16, 75 (1933), was extended to include allspice, caraway, peppermint, and pimiento and also the following direction: "Read from the extreme bottom to the bottom of the meniscus at the top of the column for allspice, peppermint and pimiento extracts."

### XVI. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

### XVII. BEERS, WINES, AND DISTILLED LIQUORS

#### *Alcohol Tables*

(1) The following changes in the alcohol tables, 19–22, to be made by the U. S. Bureau of Standards, were adopted: Tables are (a) to be based on apparent rather than true specific gravity; (b) to cover percentages from 0 to 100; (c) to show percentage of alcohol by volume at  $15.56^\circ$ ; (d) to cover samples weighed at varying temperatures (Table 22); and (e) to include a table for converting volume per cent to weight per cent.

*Beers*

(1) The following changes were made in methods: Color, Tentative (p. 134, 2), " $\frac{1}{2}$  inch cell" was substituted for " $\frac{1}{4}$  inch cell"; Specific Gravity, Official (p. 134, 3), "20/20°" was substituted for "20/4°."

(2) The following official methods were deleted, final action under suspension of the rules: Extract, Method 1 (5); Extract of Original Wort (8); Degree of Fermentation (9), and Total Acid (10).

(3) The following method for the determination of apparent extract was adopted as tentative:

**APPARENT EXTRACT**

From Table 2 ascertain the apparent extract corresponding to the sp. gr. made at 20/20°.

(4) The following methods for the determination of real extract were adopted as tentative:

**REAL EXTRACT**

(a) Evaporate on the water bath or asbestos plate 75–100 cc of sample (accurately weighed to within 0.1 g) to about  $\frac{1}{2}$  of its original volume, but do not allow the temp. to exceed 80°. Cool, make up to original weight with H<sub>2</sub>O and determine sp.gr. with a pycnometer at 20/20°. (If too much H<sub>2</sub>O has been added, the sp.gr. will be proportionately too low and a correction must be made.) Ascertain the real extract direct from Table 2 under XLII.

(b) The immersion refractometer reading of the beer at 20°—the refractometer reading of the distillate at 20° $\times$ 0.2571 = the g of extract in 100 cc of beer.

(c) If no anti-foam material was used in the determination of alcohol (5), transfer the residue quantitatively with hot H<sub>2</sub>O to a 100 cc flask. Cool, and make up to 100 cc at 20°. Determine the sp. gr. at 20/20° (3) and ascertain the extract direct from Table 2, under XLII. If 100 cc of beer was taken, make the following correction:

$$\text{Extract found} \frac{\text{sp.gr. of dealcoholized beer}}{\text{sp.gr. of beer}} = \text{g of extract in 100 g of beer.}$$

(5) The following method for the determination of extract of original wort was adopted as tentative:

**EXTRACT OF ORIGINAL WORT—TENTATIVE**

Calculate from the following formula:

$$O = \frac{A \times 2.0665 + E \times 100}{100 + (A \times 1.0665)}, \text{ in which}$$

O = Extract of original wort

A = % alcohol by weight (g per 100 g beer); and

E = real extract (6).

(6) The following method for determining real degree of fermentation was adopted as tentative:

**REAL DEGREE OF FERMENTATION OR REAL ATTENUATION—TENTATIVE**

Calculate as follows:

$$\frac{\text{Orig. ext.} - \text{real ext.} \times 100}{\text{Orig. ext.}}$$

(7) The following method for the determination of total acid was adopted as tentative:

**TOTAL ACID—TENTATIVE**

To 25 cc of beer add about 1 cc of phenolphthalein indicator soln and heat rapidly to boiling. Titrate immediately with 0.1 *N* alkali. With dark beers, dilute with  $H_2O$ . Express the results as lactic acid, g per 100 cc; 1 cc of 0.1 *N* alkali = 0.0090 g of lactic acid.

(8) The Gray-Stone method for the determination of carbonic acid was adopted as tentative (see p. 164).

(9) The following method for the determination of sulfurous acid was adopted as tentative:

**SULFUR DIOXIDE—TENTATIVE**

To 200 cc of beer (not necessarily decarbonated) in a 1000 cc distilling flask, add 250 cc of  $H_2O$  and 1 cc of  $H_3PO_4$ . Add a pinch of  $Na_2CO_3$  and distil with steam. Collect about 200 cc of the  $SO_2$ -containing distillate in a flask containing 50 cc of saturated Br water or I soln; acidify with HCl, boil to remove Br or I and precipitate with  $CaCl_2$ . Weigh as  $BaSO_4$  and report as mg of  $SO_2$  per liter.

(10) The following method for the determination of iodine reaction was adopted as tentative:

**IODINE REACTION OR UNCOVERED STARCH—TENTATIVE**

(A) 1.—Place 10 cc of beer in one test tube. 2.—In a second test tube place 0.1 *N* I soln (dissolve 12.69 g of I and 25 g of KI in  $H_2O$  and make up to 1 liter) and dilute to the same color as the beer. Slowly pour the I soln into the beer and note the color. A normal beer should not change in color. A blue color indicates starch; a purple color, amylopectine; and a reddish color, erythropectine. No change in color indicates complete conversion.

(B) *For dark beer, but applicable also to light beer.*—To 5 cc of beer in a test tube add 25 cc of alcohol. Shake thoroly. Let stand. Decant, pouring off the last trace of the beer alcohol mixture. Dissolve the precipitate (pectine) in 5 cc of  $H_2O$  and to this soln add dropwise a 0.1 *N* soln diluted 5 times. Abnormal beer gives a red or violet or blue coloration.

(11) The following method of pasteurization was adopted as tentative:

**PASTEURIZATION—TENTATIVE**

Beer heated to 58° (temp. of pasteurization) no longer contains active invertase. Take two 20 cc portions of beer, one heated to boiling, the other not heated. To each add 20 cc of a 20% sucrose soln and let stand 24 hours at room temp. Add to each soln 0.5 cc of alkaline Pb acetate soln, make up to 50 cc with  $H_2O$ , filter, and polarize. If there is an appreciable difference in rotatory power between the two samples the beer has not been pasteurized. A close agreement in rotation indicates a pasteurized beer.

(12) The following method for the determination of chlorides was adopted as tentative:

**CHLORIDES IN BEER—TENTATIVE**

Take a 100 cc sample and proceed as directed under XII, 32, 33, and 35.

(13) The following method for the determination of methyl alcohol was adopted as tentative:

## METHYL ALCOHOL (QUANTITATIVE)—TENTATIVE

Transfer to a distilling flask a quantity of sample that contains 20–25 cc of absolute alcohol and distil slowly, collecting the distillate in a 50 cc volumetric flask. When nearly to the mark, disconnect the receiver and adjust to the mark at room temp. with distilled H<sub>2</sub>O. Determine methyl alcohol as directed under 73–75.

These methods and the section on Beers will be published in a chapter entitled “Malt Beverages, Sirups, and Extracts and Brewing Materials.”

*Wines*

(1) In accordance with the footnote (p. 136) the temperature 20/4° in the specific gravity determination was changed to 15.56/15.56°, 20/20°, 25/25°.

(2) The caption “Total Acids” (p. 140, 44) was changed to “Acidity.”

(3) The following procedure for the determination of titratable acidity was adopted as tentative to be inserted as part of Method 44, p. 140, between pars. 1 and 2:

Place 200 cc of recently boiled H<sub>2</sub>O in a casserole, neutralize with phenolphthalein as indicator, add 25 cc of the wine, and titrate with 0.1 N alkali to a pink color.

(4) The following method for the determination of volatile acidity exclusive of SO<sub>2</sub> was adopted as tentative:

Determine SO<sub>2</sub> in the neutralized portion obtained under 45 by titration with standard I soln. Calculate the quantity of SO<sub>2</sub> so obtained to acetic acid, and subtract this from the volatile acid.

(5) The procedures for determining citric and malic acids described in the chapter on Fruit Products were incorporated in this chapter by cross reference.

All methods on Wines will appear in a separate chapter “Wines” in the 1935 revision of *Methods of Analysis*.

*Cordials and Liqueurs*

The following methods submitted by the associate referee were adopted as tentative and are to be inserted in the chapter on Distilled Liquors:

1

## PHYSICAL EXAMINATION

Note and record the following: (1) Appearance, whether bright or turbid and whether there is any sediment; (2) color and depth of color; (3) odor; (4) taste.

2

## SPECIFIC GRAVITY

Determine sp. gr. at 20/20° as directed in XVII, 24.

3

## ALCOHOL

(a) *By weight*.—Proceed as directed under 59.

(b) *By volume*.—Proceed as directed under 60 or 61.

4

## TOTAL SOLIDS

(a) *From the sp. gr. of the dealcoholized sample*.—Proceed as directed under 30.

(b) *By evaporation*.—Proceed as directed under XXXIV, 3.

(c) *From the insoluble residue of the dealcoholized sample.*—Restore the residue from the alcohol determination to its original volume by making the necessary evaporation or dilution. Determine the refractometer reading of the soln at 20° and obtain the corresponding percentage of dry substance. From Table 5, XLII, ascertain the sp. gr. corresponding to the percentage of dry substance found and multiply by the percentage dry substance to obtain g of total solids per 100 cc of sample. To obtain the percentage of total solids in the sample, divide the g of total solids per 100 cc by the sp. gr. of the sample, 2.

#### GLYCEROL

(a) *Products containing 5 g per 100 cc or less of total solids.*—Proceed as directed under 26 or 27.

(b) *Products containing more than 5 g per 100 cc of total solids.*—Measure into a porcelain dish such a quantity of sample (not to exceed 100 cc) as contains 25 g or less of solid matter and evaporate on the steam bath to remove alcohol. Transfer to a 500 cc Erlenmeyer flask, using such a quantity of H<sub>2</sub>O that the final volume will be approximately 100 cc, and proceed as directed in 28.

6

#### SUCROSE

##### *Method I. By polarization*

Pipet into an evaporating dish the volume of sample equivalent to 52 g, as calculated from the sp. gr. as determined under 24; exactly neutralize with normal NaOH, calculating the quantity required from the determination of acidity (7 below); and evaporate on the steam bath to remove alcohol. Transfer to a 200 cc flask and proceed as directed under XXXIV, 22 or 23, beginning "Add basic lead acetate, etc."

##### *Method II. By reducing sugars before and after inversion*

Approximate the sugar content of the sample from total solids (4 above) and pipet into a porcelain dish such a quantity of sample as will contain 5–7 g of sugars; exactly neutralize with standard NaOH soln, calculating the quantity required from the acidity, and evaporate on the steam bath to remove alcohol. Transfer to a 200 cc volumetric flask, clarify with neutral Pb acetate soln, remove the excess Pb with K oxalate, and proceed as directed under XXXIV, 28, using the method given under XXXIV, 38, for the determination of reducing sugars.

7

#### TOTAL ACIDITY

Place about 600 cc of distilled H<sub>2</sub>O in an 800 cc beaker, add about 1 cc of phenolphthalein indicator, and titrate to a pink color with 0.1 N NaOH. Add 10–20 cc of sample (unless this quantity gives the soln such a deep color that it will obscure the end point, in which case 5 cc may be used) and titrate to a pink color comparable to that of the soln before the sample was added. Calculate the acidity as g per 100 cc of sample in terms of the predominating acid present in the sample.

8

#### CHARACTERISTIC ACIDS

##### PRELIMINARY PROCEDURE

Measure out such a volume of sample as contains not more than 30 g of solid matter and not more than 200 mg of the acid to be determined, as calculated from the acidity; evaporate to about 30 cc, add 6 cc of 1 N NaOH, and let stand for at least 3 hours. Add 8 cc of 1 N H<sub>2</sub>SO<sub>4</sub>, transfer to a 250 cc volumetric flask, using 10 cc of H<sub>2</sub>O and sufficient 95% alcohol to fill the flask to the mark; mix and let stand 15 min. Filter through a thin layer of absorbent cotton, protecting the liquid against evaporation. Transfer 200 cc of the filtrate to a centrifuge bottle and proceed with the determination of the acid as directed.

9

## TARTARIC ACID

Using the material in the centrifuge bottle, proceed as directed under XXVI, 32.

10

## CITRIC ACID

Using the material in the centrifuge bottle, proceed as directed under XXVI, 35.

11

## MALIC ACID

Using the material in the centrifuge bottle, proceed as directed under XXVI.

12

## VOLATILE ESTERS

Measure 100–500 cc of sample into the distilling flask and proceed as directed under XVI, 23, collecting a volume of distillate at least twice as great as the volume of alcohol contained in the sample. Disconnect the apparatus and wash out the condenser with a little  $H_2O$ . Add about 1 cc of phenolphthalein indicator and titrate to a pink color which persists for at least 1 min., using 0.1 *N* NaOH or KOH. Add to the soln a measured excess of 25–50 cc of 0.1 *N* alkali, reflux for 1 hour, cool, and titrate the excess of alkali with 0.1 *N*  $H_2SO_4$ . Calculate the number of cc of 0.1 *N* alkali used in the saponification of the esters as ethyl acetate. 1 cc of 0.1 *N* = 8.8 mg of  $CH_3COOC_2H_5$ .

13

## GAMMA UNDECALACTONE (QUALITATIVE)

(Peach and Apricot Cordials) Note. If this procedure is to be followed, a 500 cc sample should be used above.

Make distinctly alkaline the soln obtained above (12) and evaporate to dryness on the steam bath. Take up the residue in about 25 cc of  $H_2O$ , transfer to a separatory funnel, acidify with  $H_2SO_4$  (1+1), let stand 10 min. to permit lactones to form, and extract 3 times with about 20 cc of ether. Unite the ether extracts and wash three times by shaking with 10 cc portions of normal  $Na_2CO_3$  soln. Permit the ether soln to evaporate spontaneously in a small beaker. To the residue add a few drops of  $N_2H_4 \cdot H_2O$  soln (42% in  $H_2O$ ) and mix thoroughly; if white solid matter separates out in a few minutes, gamma undecalactone is present. Allow the mixture to stand 15–20 min., place on the steam bath, and heat until the ammoniacal odor is no longer evident. Add 1 cc of normal butyl alcohol and warm until a clear soln is obtained, adding a few additional drops of the alcohol if necessary to dissolve the residue completely. Remove from the steam bath and permit the butyl alcohol to evaporate spontaneously. (This usually occurs overnight, but a longer time may be necessary if much butyl alcohol has been used.) Examine the colorless or slightly yellowish crystals under the microscope. Hydrazino- $\gamma$ -undecalactone has a characteristic odor similar to that of the lactone itself.

14

OPTICAL PROPERTIES OF HYDRAZINO- $\gamma$ -UNDECALACTONE

In ordinary light the substance is seen to consist of lath-like rods, many of them more or less split at the ends. In parallel polarized light (crossed nicols), the substance is characterized by not extinguishing sharply, most of the rods remaining essentially bright when the stage is rotated. Occasionally there are found crystals that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to the normal, are of frequent occurrence. The refractive indices, as determined by the immersion method, are as follows:  $\alpha = 1.483$  (not common);  $\beta = 1.525$  (most frequently occurring of the indices and shown lengthwise on rods);  $\gamma = 1.555$  (occurring crosswise on rods which show straight extinction and negative elongation); all  $\pm 0.003$ .

15

## METHYL ALCOHOL

Measure into a distilling flask such a quantity of sample as contains 20–25 cc of absolute alcohol, add sufficient  $H_2O$  to make the total volume about 100 cc, and distil, collecting about 50 cc of distillate. To the distillate add 4 g of NaCl for each 10 cc of  $H_2O$ , and allow to stand several hours to reach the saturation point. Transfer to a separatory funnel, using about 10 cc of saturated NaCl soln to wash out the container, and shake with 25 cc of petroleum ether. When the separation is complete, transfer the  $H_2O$  soln to a second separatory funnel containing 25 cc of petroleum ether; shake, and transfer the  $H_2O$  soln to a third separatory funnel, also containing 25 cc of petroleum ether; shake and when the separation is complete, drain off the  $H_2O$  soln into a 200 cc distilling flask. In the meantime, add to the first funnel 25 cc of saturated salt soln and follow the sample through with this soln, finally adding the washings to the sample soln in the distilling flask. Repeat this operation with a second 25 cc portion of saturated salt soln, finally adding this also to the distilling flask. Now distil the mixture into a 50 cc volumetric flask, using a suitable adaptor. When 48–49 cc has distilled over, disconnect the apparatus and fill the flask to the mark with distilled  $H_2O$ . Mix, and determine methyl alcohol as directed under Distilled Liquors.

16

## ASH

Proceed as directed under XXVII, 8, using 25 cc of sample, the temp. of ashing not to exceed  $525^\circ$ .

17

## SOLUBLE AND INSOLUBLE ASH

Proceed as directed under XXXIV, 12.

18

## ALKALINITY OF SOLUBLE ASH

Proceed as directed under XXXIV, 13.

19

## ALKALINITY OF INSOLUBLE ASH

Proceed as directed under XXXIV, 14.

20

## PHOSPHORIC ACID

Evaporate 25 cc of sample to a sirupy consistency on the steam bath; add 7.5 cc of  $Mg(NO_3)_2$  soln, II, 5 (e); mix thoroly, continue the evaporation as far as possible on the steam bath, and proceed as directed under XII, 26, beginning with, "heat on an electric hot plate at  $180^\circ$ ."

21

## BENZALDEHYDE

*Phenylhydrazine reagent.*—Add 1.5 cc of glacial acetic acid and 1 cc of newly distilled phenylhydrazine to 20 cc of  $H_2O$  and filter through a moistened double white ribbon filter.

Measure into a distilling flask such a quantity of sample as contains 30 cc of absolute alcohol, dilute to such a volume that the mixture will contain 300 cc of  $H_2O$  in addition to that required to dissolve the sugar present (1 g of sugar requires 0.5 cc of  $H_2O$ ), and distil off 300 cc into a 500 cc Erlenmeyer flask. Add 10 cc of the reagent and shake for 5 min. Filter on a Gooch with a thin mat, and wash with  $H_2O$  and finally with two 10 cc portions of 10% alcohol. Dry in a vacuum desiccator over  $H_2SO_4$  for 24 hours, excluding light, or at  $70^\circ$  under 100 mm or less of pressure for 2 hours. Wt. of precipitate  $\times 0.5408$  = benzaldehyde.

22

## CAMEL

Proceed as directed under XVII

23

## COAL TAR COLORS

Proceed as directed under XXI.



24

**ALDEHYDES**

Measure 100–200 cc of sample into a distillation flask. If the solid content is 25 g per 100 cc or less, add 12.5–25 cc of  $H_2O$ ; if greater than 25 g per 100 cc, add 5 cc of  $H_2O$  for each 10 g of solid matter present, and distil slowly, collecting a volume of distillate equal to that of the sample, and proceed as directed under 67.

25

**FURFURAL**

Treat a portion of the prepared distillate, 24, as directed under XVII, 69.

26

**FUSEL OIL**

Treat 50 cc of the prepared distillate, 24, as directed under XVII, 71.

*Whiskey, Rum, and Brandy*

(1) The paraldehyde test for caramel (p. 148, 80) was deleted, and the zinc acetate method developed by Valaer and Mallory, *This Journal*, 18, 75 (1935), was adopted as official (first action.)

(2) The table submitted by the associate referee was substituted for Table 6.

(3) The method for the detection and determination of methyl alcohol, published previously in *This Journal*, 18, 477 (1935), was adopted as tentative after being modified to provide: (a) that the aliquot of sample contain not more than 0.160 g of methyl alcohol; (b) that 25 cc of 95% alcohol be introduced into the reaction flask immediately before the sample is introduced; and (3) that 25 cc of wash solution be used in the receiving flask instead of 25 cc of absolute alcohol.

This method will appear in the Chapter on Distilled Liquors and by cross reference in the Chapters on Wines and on Malt Beverages, Sirups, and Extracts and Brewing Materials.

**XVIII. COFFEE AND TEA**

No additions, deletions, or other changes.

**XIX. CACAO BEAN AND ITS PRODUCTS**

(1) The tentative micro method for detection of shell (p. 162, 30) was dropped.

(2) The tentative method for separation and preparation of fat in cacao products (p. 159, 16) was revised as follows: In line 1, "2 to 10 g" was changed to "10–40 g."

**XX. CEREAL FOODS**

(1) The following methods taken from "Cereal Laboratory Methods" were adopted as tentative: Original ash of phosphated and self-rising flour (Gustafson method), and total carbon dioxide in self-rising flour.

**ORIGINAL ASH OF PHOSPHATED AND SELF-RISING FLOUR***Gustafson Method*

To 20–25 g of the flour in a metal centrifuge tube (cup 2 in. in diameter, 6 in. deep), add sufficient  $CCl_4$  to fill the tube to within 1 in. of the top (about 250 cc). Centrifuge 5–7 min. at a speed of 1,600 r.p.m., and allow the centrifuge to come to

rest slowly. Carefully skim off the flour, which is now in a compact layer on the surface of the  $\text{CCl}_4$ , with a large tablespoon, recovering as much of the flour as is possible in one spoonful. (With care, about 90% of the original flour may be recovered.) Allow the wet flour to dry overnight and proceed as directed under **XX, 6**. (The carbon tetrachloride may be filtered, distilled, and used again.)

#### TOTAL CARBON DIOXIDE IN SELF-RISING FLOUR

Use 17 g of flour and 40 cc of  $\text{H}_2\text{SO}_4$  (1+5) and proceed as directed under **XV, 8-10** as far as the calculation. Calculate as follows: Subtract the volume of acid used from the total buret reading and correct for temp. and pressure. Divide the corrected reading by 100 to obtain the percentage of  $\text{CO}_2$  (by weight) in the self-rising flour. To convert  $\text{CO}_2$  to  $\text{NaHCO}_3$ , multiply  $\text{CO}_2$  by the experimentally determined factor 2.01.

(2) Method 1, tentative for the determination of unsaponifiable residue (p. 170, 28), was dropped.

(3) Method 1, tentative for the determination of bleaching chlorine (p. 173, 38), was dropped.

(4) The following precautionary note was added to the method for the determination of chlorides in ash as  $\text{NaCl}$  (p. 181, 64): "This sodium chloride value deducted from the total ash does not give  $\text{NaCl}$ -free ash."

(5) Method I for the determination of fat (p. 178, 53) was dropped.

(6) Method II for the determination of fat by acid hydrolysis in baked cereal products and macaroni products (p. 178, 54 and p. 181, 65) was made official (final action).

(7) The method for the extraction and identification of added color in macaroni products (p. 181, 72) was dropped (final action under suspension of the rules).

(8) The following method for the extraction, separation and identification of added color in macaroni products was adopted as official (first action):

#### EXTRACTION, SEPARATION, AND IDENTIFICATION OF COLORING MATTER IN MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS

Place approximately 500 g of the coarsely ground sample (depending upon the quantity of color present) in a liter Erlenmeyer flask, add about 700 cc of 80% alcohol, and shake at intervals for 24 hours or until the color is imparted to the solvent. Place the flask with contents in the refrigerator overnight to permit dissolved protein matter to precipitate out. Filter, and evaporate the filtrate to 100 cc. Add to the filtrate about  $\frac{1}{4}$  volume of 25% salt soln and a slight excess of ammonia; cool, and transfer to a separatory funnel. Extract this mixture with an equal volume of ligroin or petroleum ether; separate the lower layer and repeat extractions with additional portions of the solvent, until no more color is extracted. Reserve the lower layer, if colored, for further treatment; if colorless, discard. Combine the petroleum ether extracts and wash with several small portions of ammonia water (1+50) to remove any material mechanically adhering to the solvent. This ethereal soln will contain the fats, and also may contain the oil-soluble coal tar dyes, which may be identified by the procedure under (1). If colored, immediately acidify the alkaline aqueous soln freed from fat and oil-soluble coal tar dyes with acetic acid and extract in 25 cc portions with two 50 cc volumes of ether. The solvent, if colored, may contain turmeric, annatto, and a trace of saffron. For their identifica-

tions use procedure (2). If the original aqueous soln freed from ether-soluble colors should still be colored, water-soluble dyes may be suspected, in which case the following procedure is recommended: Extract the aqueous soln with 50 cc portions of amyl alcohol to remove the balance of saffron, as well as the common orange dyes (S & J numbers 85, 86, 13) as also martius yellow. For their separation proceed as directed under XXI. Draw off the lower aqueous layer, which, if colored, may contain naphthol yellow S and tartrazine and also sunset yellow. Extract these dyes with amyl alcohol after acidifying the soln with HCl to make approximately 1 N. Remove tartrazine from the solvent with 0.25 N HCl. Sunset yellow will also be removed at this stage with a slightly lower acid concentration, and naphthol yellow S from a nearly neutral soln. Confirm with wet and spot reactions. The extracted solns are usually very dilute, therefore it is advisable to concentrate by evaporation over a steam bath, and if not clear, to add about 5 cc of 25% salt soln to break up slight emulsions by precipitating the protein matter, filtering, and testing the filtrate, by dyeing and coupling. This coupling test is carried out as follows: Treat about 10 cc of the filtered soln with excess of bromine, destroy the excess with a saturated soln of hydrazine sulfate, and immediately pour into a sodium carbonate soln of alpha naphthol. In the presence of tartrazine or sunset yellow a pink color will be produced. It is advisable to run a blank determination on the above test for comparison.

(1) Extract the original petroleum ether extract with two or three 10 cc portions of a mixture consisting of 1 part of HCl and 5 parts of acetic acid.

In the presence of S & J numbers 7 and 16, yellow OB or yellow AB, a pink or red color is obtained. Test a small portion of this acid extract with a few drops of  $\text{SnCl}_2$ , which in the presence of the above dyes will be either decolorized or produce a decided fading. Dilute the balance of the acid extract with  $\text{H}_2\text{O}$ , make slightly alkaline, and extract the color with petroleum ether. Wash the solvent with 2-5 cc portions of  $\text{H}_2\text{O}$  to remove excess of alkali. Test approximately 5 cc portion of the petroleum ether extract with formaldehyde and acetic anhydride as directed under XXI, 9(a). Evaporate another 5 cc portion of the petroleum ether extract to dryness in a small evaporating dish and observe spot tests with HCl and  $\text{H}_2\text{SO}_4$ . Evaporate to dryness the balance of the petroleum ether extract in a small casserole and dissolve the residue in dilute alcohol. Dye some silk strands, preferably using a slightly alkaline soln. Compare the spot tests obtained with Table II, XXI. If they do not agree with the tables, a mixture of dyes may be present, which will necessitate a separation according to the pH concentration.

The remaining coloring matters in the ligroin extract may be due to the natural coloring matter of wheat, or to the coloring matter of egg. The coloring principle of egg yolk, lutein, when heated with alcoholic ferric chloride, will produce a green coloration. However, this test is not specific for lutein, as carotin and xanthophyll give similar reactions.

(2) Wash the ether extract with 5 cc portions of  $\text{H}_2\text{O}$  to remove excess of acid. To remove annatto and the traces of saffron, wash successively with 20 cc portions of 5%  $\text{NaHCO}_3$  soln. Divide this alkaline soln into two portions. Heat one portion to  $60^\circ$  on the steam bath and dye the color on unmordanted cotton, and compare spot tests with a standard. Acidify the remaining portion of the alkaline annatto soln with acetic acid and re-extract with ether. Divide the ethereal extract into two small casseroles and evaporate to dryness. Dissolve the contents of one casserole in 10 cc of ammonia water (1+9) and impregnate it on a strip of cotton or filter paper. An orange yellow to an orange red coloration is obtained depending upon the amount of dye present. Dry the filter paper or cotton, and add a drop of 40%  $\text{SnCl}_2$ , and again dry. In the presence of annatto a purple stain is produced. Spot the contents of the other casserole with  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , when a blue and a green-

ish blue color are obtained. Transfer two portions (of about 10 cc each) of the original ether extract from which annatto has been removed, into test tubes and treat same with an equal volume of 10% NaOH and an equal volume of HCl (1+1), respectively. In presence of turmeric (curcuma) the alkaline soln will be of reddish brown, while the acid solution will be a red. Turmeric can further be confirmed by its behavior with boric acid. Apply this test as follows: Shake a portion of the original ether extract with an equal volume of 70% alcohol and to this add 1/10 volume of HCl, mix, and divide the soln equally into two test tubes. To one tube add a few crystals of boric acid and shake. Use the other tube as a control. In the presence of turmeric, a red color will be produced after a short time.

(3) To separate and identify saffron and the orange coal tar dyes, dilute the amyl alcohol extract with two volumes of petroleum ether and extract the mixed dyes with several 10 cc portions of  $H_2O$ . To a smaller portion of this aqueous extract add 1/10 volume of glacial acetic acid and add a few mg of dry sodium hyposulfite to reduce all the azo dyes. This treatment will not affect the saffron, which can then be reextracted by amyl alcohol. After washing the solvent repeatedly with small portions of  $H_2O$  (to remove decomposition products) evaporate to dryness, and confirm the presence of saffron by spot tests. The remainder of the color soln after addition of salt and acetic acid is re-extracted with amyl alcohol and later fractionated from the solvent for S & J numbers 85, 86, 13, by 5%  $Na_2CO_3$  soln. Martius yellow if present will still remain in the amyl alcohol and petroleum ether after the removal of the saffron and oranges. In order to prove its presence, evaporate the solvent to dryness and dissolve the residue with 10 cc ammonia (1+9). Divide into two test tubes. Add carefully to one a few crystals of sodium hyposulfite. The presence of martius yellow will manifest itself by the formation of a pink soln. To check its presence use the other subdivision for dyeing, spotting, etc.

(9) The tentative method for the detection of the presence of whole egg or commercial yolk solids, and the method for the determination of egg solids (p. 182, 73, 74) were dropped.

(10) The following method (Walters) for the rapid determination of ash in flour was adopted as tentative:

#### ASH IN FLOUR

##### *Quick Ashing Method*

#### SOLUTIONS

Dissolve in 40%  $C_2H_5OH$  sufficient of the nitrate chosen to yield approximately 0.015 g of oxide upon ignition.

|              |                         |                          |
|--------------|-------------------------|--------------------------|
| 1.9935 g La  | $(NO_3)_3 \cdot 6H_2O$  | yields 0.015 g $La_2O_3$ |
| 1.8918 g Ce  | $(NO_3)_4 \cdot 12H_2O$ | yields 0.015 g $CeO_2$   |
| 1.5681 g Th. | $(NO_3)_4 \cdot 4H_2O$  | yields 0.015 g $ThO_2$   |
| 2.5441 g Y   | $(NO_3)_3 \cdot 6H_2O$  | yields 0.015 g $Y_2O_3$  |

Run a blank on the nitrate soln to determine the exact quantity of oxide present.

#### DETERMINATION

Weigh 3-5 g of flour into a dish approximately 65 mm in diameter and 25 mm in depth. Add with a pipet exactly 10 cc of the nitrate soln. Stir with a glass rod until all the flour is moistened. Clean the rod with a small piece of ashless filter paper and add the latter to the sample. Burn off excess alcohol. Transfer the dish to a muffle furnace that is already at  $850^\circ$ . Leave door of furnace open until flaming has ceased, then close. When the ash is entirely white (30-45 min.) remove the

dishes to desiccator, cool, and weigh. Weight of crude ash—weight of blank = true weight of ash.

(11) The method submitted by the associate referee for the detection of benzoyl peroxide bleach in flour was adopted as tentative. This method is a modification of the method published in *This Journal*, 18, 493 (1935).

(12) The method for the determination of the diastatic value of flour published in *This Journal*, 16, 501 (1933); 17, 65, 397 (1934); 18, 76 (1935) was adopted as official (final action).

(13) The two methods submitted by the associate referee for the determination of milk solids in bread were adopted as tentative. The method based on fat content has been published, *This Journal*, 18, 574 (1935). The method based on the citric acid content follows:

#### MILK SOLIDS IN BREAD

##### *Citric acid method*

To a weight of air-dried bread equivalent to 77.7 g of moisture-free bread in a 500 cc volumetric flask, add 400 cc of a mixture containing 25 cc normal  $\text{H}_2\text{SO}_4$ , 20 cc of a 20% phosphotungstic acid soln, 55 cc of  $\text{H}_2\text{O}$ , and sufficient 95% alcohol to make 500 cc. Shake for 5 min., make to mark, and allow to stand overnight. Readjust to mark with 95% alcohol, shake 5 min. and filter with suction on paper in a 12 cm Büchner funnel. Transfer 325 cc of the filtrate to a centrifuge bottle, add 30 cc of Pb acetate soln (75 g of the salt plus 1 cc of glacial acetic acid diluted to 250 cc with  $\text{H}_2\text{O}$ ), shake 5 min. and centrifuge at about 900 r.p.m. for 15 min. Decant the supernatant liquid (disregard turbidity), allow to drain, transfer the residue with about 150 cc of  $\text{H}_2\text{O}$  to a 250 cc volumetric flask, and thoroughly saturate with hydrogen sulfide. Make to mark with  $\text{H}_2\text{O}$ , shake thoroughly, and filter through a large folded filter. Evaporate 200 cc of the clear filtrate in a 500 cc Erlenmeyer flask over free flame to about 75 cc. Cool to 45–50°, and add 10 cc of  $\text{H}_2\text{SO}_4$  (1+1), 5 cc of potassium bromide and 15 cc of permanganate soln (5 g of potassium permanganate diluted to 100 cc). After about 2 min., stopper the Erlenmeyer, shake vigorously, and allow to stand 3 min. longer. Add 20 cc of ferrous sulfate soln (40 g of the salt plus 1 cc of  $\text{H}_2\text{SO}_4$  diluted to 100 cc with  $\text{H}_2\text{O}$ ), cool to about 15°, and shake vigorously until the pentabromacetone has crystallized (lace-like deposit on the walls of the flask). Place in a refrigerator at about 15° overnight. Avoid a temp. of less than 15° since at lower temp. there is the tendency of the pentabromacetone to freeze on the sides of the flask. Filter and dry the pentabromacetone as directed under XXVI, p. 273. If the drying is done by aspiration, 20 min. should be sufficient time. It is also advisable to cool the air current by placing the vessel containing the  $\text{H}_2\text{SO}_4$  into ice cold  $\text{H}_2\text{O}$ . Weight of pentabromacetone in g— $0.004 \text{ g} \times 75$  = per cent whole milk solids in moisture-free bread.

(14) The following methods for the examination of brewing materials, to be inserted in the new chapter on Malt Beverages, Sirups, and Extracts, and Brewing Materials, were adopted as tentative:

#### MALT<sup>1</sup>

##### PREPARATION OF SAMPLES

*Bulk Malt.*—Take at least 6 probes with a double-tubed, separate-compartment grain trier from different parts of the shipment.

<sup>1</sup> The methods under Malt are essentially those adopted by the American Society of Brewing Chemists.

**Bags.**—Take samples from at least 2% of the bags with a probe.

Mix these samples immediately and thoroly and place in a large glass or metal container. Take 2 sub-samples of about 1 pound each and place each in an air-tight container (preferably tin with a screwtype cover) or in a glass-stoppered bottle.

#### BUSHEL WEIGHT

Place the malt into the filling hopper of a Winchester bushel weight tester, open the slide underneath, and fill the measuring cylinder to overflowing. Without jarring, level off with a straight-edge, making one forward stroke consisting of three distinct zig-zag motions. Weigh to the nearest one-quarter pound.

#### LENGTH OF ACROSPIRE

Reduce the sample by quartering until about 200 kernels remain in opposite quarters. Count off 100 kernels and cut longitudinally into halves. Record length as 0- $\frac{1}{4}$ ,  $\frac{1}{4}$ - $\frac{1}{2}$ ;  $\frac{1}{2}$ - $\frac{3}{4}$ ,  $\frac{3}{4}$ -1, over 1, in percentage totaling 100%.

At the same time determine the mealiness.

#### MEALINESS

Pierce the starch body and record as mealy, half glassy, glassy in percentage, totaling 100%.

#### 1000 KERNEL WEIGHT

Reduce the sample by quartering until about 500-600 kernels remain in opposite quarters. Count off 500 kernels and weigh to nearest 0.1 g. Calculate the results to 1000 kernels on "as is" and on dry basis.

#### ASSORTMENT

Determine uniformity of size of kernels by passing 100 g through a barley grader consisting of 5 different sized screens. Shake for 3 min. at 300 r.p.m. Weigh quantities remaining on the different screens to nearest 0.1 g and report as on 8/64 in. screen, 7/64 in. screen, 6 $\frac{1}{2}$ /64 in. screen, 5 $\frac{1}{2}$ /64 in. screen, 5/64 in. screen, and through 5/64, in percentage, totaling 100%.

#### MOLD

Determine the presence or absence of mold by visual inspection and report as "none," "trace," etc.

#### FOREIGN SEEDS AND BROKEN KERNELS

Weigh off 50 g of the malt sample. Pick out foreign seeds and broken kernels, classify, and report separately (per cent).

#### MOISTURE

Use about 5 g of sample. Use a drying oven provided with one shelf and an automatic temp. control and keep the temp. within 0.5°. Test out the oven at 103-104° and dry duplicate ground samples for 3 hours. Weigh, and redry for 1 hour longer. If the second weight varies from the first more than 0.1%, raise the temp. of the oven 1°. (Samples should not be heated above 106°, and moisture content should be within 0.1%.) Use glass bottles or Al dishes 40 mm in diameter with tight-fitting covers. After testing the oven, weigh out 55 g of malt and grind in a mill as directed under Extract, using 50 g for the extract determination and about 5 g for moisture. Report moisture with one decimal nearest to 0.1%.

#### EXTRACT

Use a cone type (Miag-Seck) mill set for finely ground malt (300 r.p.m.) and determine the setting of the mill by the composition of a well modified malt. Grind 50 g of malt, mix well, and test by shaking by hand for 5 min. in a series of 0.5 in.

half-height U. S. Standard sieves consisting of a set of 6 sieves with pan and cover designated by numbers 10, 14, 18, 30, 60 and 100, each 8 in. in diameter. Use a horizontal shaking motion with tapping upon the table top every 15 sec. Adjust the setting of the mill so that the sum of the ground malt portions remaining on sieves 10, 14, 18, and 30 is between 4.5 and 5.5 g (equal to 9–11%).

**Laboratory Mashing Apparatus.**—The mash beakers and stirrers may be made of either pure nickel or brass, but both should be of the same metal. The ground malt previously collected in the weighed mash beaker should weigh 50 g. Mix the malt with 200 ml of H<sub>2</sub>O of 46°, stir with a glass rod, and wash off wall and glass rod with a little H<sub>2</sub>O. Place the beaker in the water bath of the mashing apparatus, adjust stirrer (80–100 r.p.m.) and maintain temp. of 45° for 30 min. Raise temp. 1° each minute until 70° is reached. Add 100 ml of H<sub>2</sub>O of 70° and mash at this temp. for 60 min. While mashing note odor of mash and record as “aromatic,” “slightly aromatic,” or “unpleasant” (green, musty, stale).

**Conversion.**—5 min. after the mash has been held at 70°, take out a drop of the mash with a thin glass rod and place on absorbent gypsum plate, adding 1 drop of 0.01 N I soln. When the test drop shows only a yellow stain, conversion is complete. If necessary, repeat and record as “less than 5 min.,” “5–7 min.,” etc.

**Cooling.**—After holding the mash for 60 min. at 70°, cool in 10–15 min. to room temp. Stop machine, remove beaker after carefully washing off mash adhering to stirrer, dry beaker, and weigh contents of beaker to 450 g by adding distilled H<sub>2</sub>O. Mix the contents of the beaker with a glass rod and filter through a Schleicher-Schuell 32 cm fluted filter paper No. 560, or No. 597, 32 cm filter, analyst to flute same, or Delta-Dueren 32 cm filter No. 314½ or 314, 32 cm, to be fluted by analyst. The filter paper should not extend beyond the edge of the funnel. Pour the entire contents of the beaker on the filter, cover with a watch-glass, and after 100 cc has passed through the filter, return same. Record the speed of filtration as normal if filtration is completed in 1 hour. Note if filtrate is “clear” “slightly hazy” or “hazy.”

**Specific gravity.**—Determine sp. gr. with any pycnometer but preferably use a Reischauer pycnometer about 15 cm high, neck 9 cm, and capacity about 50 cc. Standardize pycnometer with H<sub>2</sub>O of 20°. Calculate the sp. gr. to the 5th decimal place. (Make no calculation of the sp. gr. in vacuum.) Allow the pycnometer to remain in a constant water bath for 20 min. The extract of the malt-wort is taken from the Plato Tables at 20°, XLII, 2.

#### CALCULATION OF EXTRACT

Total extract =  $\frac{\text{Extract (800 + W)}}{100 - \text{Extract}}$ , where extract = extract from Plato's ta-

bles; W = % moisture in malt; and extract in dry substance =  $\frac{\text{Extract in Malt}}{100 - \text{Moisture}}$ .

#### COLOR OF WORT

Use a Lovibond tintometer, ½-inch Cell, Series 52 (brewers' type). Standard Daylight Lamp (see A.S.T.M. tentative standards for 1933).

#### DIASTATIC POWER

Wash all glassware with acid-cleaning soln, then rinse with ordinary tap H<sub>2</sub>O not less than 4 times, and finally rinse with distilled H<sub>2</sub>O at least twice. Thoroughly dry the digestion flasks.

#### REAGENTS

**Soxhlet's modification of Fehling's soln.**—Prepare by mixing immediately before use equal volumes of (a) and (b).

(a) *Copper sulfate soln.*—Dissolve 34.639 g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in  $\text{H}_2\text{O}$ , dilute to 500 ml, and filter through prepared asbestos.

(b) *Alkaline tartrate soln.*—Dissolve 173 g of Rochelle salt and 50 g of  $\text{NaOH}$  in  $\text{H}_2\text{O}$ , dilute to 500 ml, allow to stand for 2 days, and filter through prepared asbestos.

Check the Fehling's soln from time to time by estimating its oxidizing value against a standard soln of invert sugar according to customary analytical procedure.

#### PREPARATION OF STARCH SOLUTION

Have the final concentration represent 2 g of soluble starch (weighed on a dry basis), in 100 ml of soln. (Use starch of such quality and grade that its solubility will be at least 1:50 in hot  $\text{H}_2\text{O}$ , contain no dextrines, contain less than  $\frac{1}{4}$  of 1% reducing substances calculated as maltose, and have a moisture content of approximately 10–12%. A freshly made 2% soln shall have a pH between 4.5 and 4.7 without adjustment by the use of a buffer. It shall be originally subject to the approval of a body designated for the purpose. Subsequent batches of starch shall, when tested on a malt of approximately 100° Lintner (dry basis) and having other characteristics as specified under the determination of extract in malt, show a variation no greater than  $\pm 3^\circ$  Lintner from the value obtained using the original starch in a parallel determination. Further additional batches of starch when purchased shall be tested in parallel with the starch in use. No variation greater than  $\pm 3^\circ$  Lintner will be permitted. In no case shall a cumulative correction as referred to the original starch approved above amount to more than  $5^\circ$  Lintner.) Macerate the starch with a small amount of cold freshly distilled  $\text{H}_2\text{O}$  sufficient to form a smooth thin paste (not over 5% of final volume). Pour this, with constant stirring, into boiling freshly distilled  $\text{H}_2\text{O}$  representing not less than approximately 75% of the final volume of the starch soln at such a rate that boiling does not cease. Continue boiling for 2 min. from the time that the thin paste is completely introduced. Quickly add an additional 10% of the final volume of cold freshly distilled  $\text{H}_2\text{O}$  to the beaker and transfer the mixture quantitatively to a glass-stoppered volumetric flask, mix by inverting the flask, wash down the neck of the flask, and cool the whole to  $20^\circ$  before adding the buffer soln. Add 2 ml of standard acetate buffer soln (dissolve 68 g of sodium acetate in 500 ml of normal acetic acid and make up to 1 liter with distilled  $\text{H}_2\text{O}$ ) for each 100 ml of the final volume of starch soln and make up the whole to the mark. Mix again by inverting the flask and keep tightly stoppered at  $20^\circ$  until used.

#### PREPARATION OF MALT INFUSION

Grind separately not over 25.5 g of malt as directed under Extract. Collect the finely ground malt in a mash beaker. Carefully brush malt particles remaining in the mill into the mash beaker. Without delay, place the mash beaker with its contents on the balance and adjust the contents to 25 g ( $\pm 0.05$  g). Transfer quantitatively the 25 g to the container (about 1 liter) in which the infusion is to be made. Add 500 ml of freshly distilled  $\text{H}_2\text{O}$  and close the container. Let the infusion stand for 2.5 hours at  $20^\circ$  ( $\pm 0.2^\circ$ ) and agitate by rotation at 20 min. intervals. Take care that in the agitation of the malt suspension as small a quantity as possible of the grist is left adhering to the inner surface of the flask above the level of the  $\text{H}_2\text{O}$ . Mixing by inverting the flask is specifically cautioned against. Gentle whirling of the contents without splashing on the sides of the container has been found to give sufficient mixing. Filter the infusion by transferring the entire charge onto a 30–32 cm fluted filter (CS and S No. 588) contained in a 175 mm funnel. Return the first 50 ml of the filtrate to the filter. Collect the filtrate until 3 hours shall have elapsed from the time the  $\text{H}_2\text{O}$  and ground malt were first mixed. Prevent evaporation dur-



ing the filtration period as far as possible by placing a watch-glass over the funnel and some suitable cover around the stem of the funnel, resting on the neck of the receiver.

#### DETERMINATION

Dilute immediately 20 ml of the above infusion to 100 ml at 20°, transfer 10 ml of this infusion to a 200 ml volumetric flask and bring to 20°. If the diastatic power of the malt being examined be 135° Lintner or above, make (or repeat) the determination, using a 250 ml volumetric flask at this point and 200 ml of the buffered starch soln. Multiply the diastatic power as computed under Determination of Blank Correction by 1.25. Add 100 ml of buffered starch soln from a fast flowing pipet all at 20°. Mix the solns by rotating the flask during the addition. Maintain the "starch-infusion" mixture at 20° ( $\pm 0.2^\circ$ ) for exactly 30 min. from the time addition of the starch soln was begun. Add 20 ml of 0.25 N NaOH rapidly for each 100 ml of starch soln and mix the whole thoroly by whirling the flask. Make to the mark at 20° and mix well.

Boil 10 ml of the Fehling soln and 10 ml of H<sub>2</sub>O in a small flask with narrow neck (200 ml Erlenmeyer).

Add from a buret about  $\frac{1}{2}$  of the amount of the above digested starch soln probably required and boil 15–20 seconds, rotating constantly. Remove from flame. If still decidedly blue, add more soln, boil about 10 seconds, and again observe color. When the blue color has been almost discharged, and after boiling gently for about 2 min., add 3 drops of a 1% aqueous methylene blue soln. Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges the blue color. (It becomes violet-lavender as end point nears.)

Interrupt the boiling as little as possible after the indicator has been added, so that the flask remains filled with steam, preventing much access of air. (Upon cooling the blue color usually returns.)

To compute the uncorrected diastatic power, divide into 4000 the number of ml of the digested starch soln required to reach the above end point.

#### DETERMINATION OF BLANK CORRECTION

Prepare a blank by proceeding exactly as described under Determination, except to add the NaOH to the malt infusion before adding the starch soln. Add to 10 ml of the Fehling soln and 10 ml of H<sub>2</sub>O a volume of this blank equal to the volume of digested starch soln required in the above determination. Boil and again determine the end point, using the digested starch soln, as directed under Determination.

The difference between the amount of digested starch used in the first determination and that used in the second determination represents the influence of the starch.

To determine the corrected diastatic power, multiply the number of ml required in the second determination by 4000 and divide this by the square of the number of ml required in the first determination. To convert this to "dry basis" divide the figure so found by (100 minus per cent moisture). Report as degrees Lintner (dry basis).

### PREPARED CORN OR RICE PRODUCTS (FLAKED CORN OR FLAKED RICE)

#### MOISTURE

Weigh out about 5 g in a glass weighing bottle or Al dish with tightly fitting cover, 40 mm in diameter, and dry at 103–104° for 4 hours. Record results to nearest first decimal.

**FAT**

Extract the sample from the moisture determination with anhydrous ether for 5-6 hours, distil off the ether, and dry in an oven for 1 hour. Cool, and weigh.

**EXTRACT**

To 30 g of finely ground barley-malt contained in a weighed mash beaker, add 200 ml of distilled H<sub>2</sub>O of 46°. Mix malt and H<sub>2</sub>O with a glass rod, washing off rod and sides of beaker with a little distilled H<sub>2</sub>O. Maintain the temp. of 45° with constant stirring, preferably in a mashing machine for 30 min. If no mashing apparatus is available, place beaker on a wire screen contained in a water-bath. Raise the temp. 1° per min. until 67° is reached. Add 20 g of the prepared flaked corn or rice product, mix thoroughly, and hold for 30 min. at 67°. Warm up to 70° in 6 min. Hold until saccharified, testing every 3 min. by taking a small portion of the mash with a thin glass rod and placing this on a gypsum plate together with a drop of 0.01N I soln. A yellow coloration indicates complete inversion. Note the time after reaching 70° until mash is completely inverted. Hold altogether at 70° for 60 min. Cool to room temp., remove beaker from bath, wash off thermometer or stirrer with a little distilled H<sub>2</sub>O, dry beaker, and weigh contents to 450 g. Stir contents of beaker thoroughly and filter through a fluted filter. Pour the first 100 cc of filtrate back on the filter, collecting the entire filtrate in a 500 cc Erlenmeyer flask. Determine the sp. gr. with a pycnometer, preferably a Reischauer pycnometer at 20°, and find corresponding extract in the Plato Tables, XLII, 2.

Calculate the extract as follows:

$$\text{Total extract} = \frac{\text{Extract} \times (800 + W \text{ in } 60 \text{ g of malt} + W \text{ in } 40 \text{ g of Flakes})}{100 - \text{Extract}}, \text{ where}$$

Extract = extract from Plato's Tables;

W = Moisture;

$$\text{Extract in flakes} = \frac{\text{Total extract} - \text{extract in } 60 \text{ g of malt}}{40}.$$

**CORN GRITS, CORN MEAL, BREWERS' RICE****PREPARATION OF SAMPLE**

If necessary, grind to fairly fine consistency.

**MOISTURE**

Weigh out about 5 g in a glass weighing bottle or Al dish 40 mm in diameter with a tightly fitting cover, and dry at 103-104° for 4 hours. Record results to nearest first decimal.

**FAT**

Extract the sample from the moisture determination with anhydrous ether for 5-6 hours, distil off ether, and dry in oven for 1 hour. Cool, and weigh.

**EXTRACT**

Boil for 30 min. 20 g of finely ground sample in a mash beaker with 200 ml of H<sub>2</sub>O, stirring with a glass rod and replacing the evaporated H<sub>2</sub>O. Cool to 46°, and add 30 g of crushed malt. Mix, and wash off glass rod and sides of beaker with a little H<sub>2</sub>O. Maintain the temp. of 45° for 30 min. with constant stirring, preferably in a mashing apparatus. If no mashing apparatus is available, place beaker on a wire screen contained in a water bath. Raise temp. 1° every minute until 70° is reached, and hold at this temp. until saccharified, testing very 3 min. by taking a small portion of the mash with a thin glass rod and placing this on a gypsum plate together with a drop of 0.01N I soln. A yellow coloration indicates complete inver-

sion. Note the time after reaching 70° until mash is inverted. Hold altogether at 70° for 60 min. Cool to room temp., remove beaker from bath, wash off stirrer and thermometer with a little distilled H<sub>2</sub>O, dry beaker, and weigh contents of beaker to 450 g. Stir with a glass rod and filter through a fluted filter paper. Pour the first 100 cc of filtrate back on the filter, collecting the entire filtrate in a 500 cc Erlenmeyer flask. Determine the sp. gr. with a Reischauer pycnometer at 20° and find corresponding extract in the Plato Tables, XLII, 2.

Calculate the extract as directed for flakes.

### REFINED GRITS AND REFINED FLAKES

Proceed as directed under the examination of corn grits except to boil for 5 min. only in the determination of the extract.

### XXI. COLORING MATTERS IN FOODS

Numerous changes were made in this chapter to provide for the additions of three colors: Sunset yellow FCF, ponceau SX, and brilliant blue FCF.

### XXII. DAIRY PRODUCTS

(1) The methods for the determination of ash and total chlorides in cheese published in *This Journal*, 18, 401 (1935) were adopted as tentative.

(2) The tentative method for the determination of lactose in milk, p. 216, was amended as follows: In 12(b), line 1, change "20 cc" to "200 cc"; in 13, second par., line 3 insert after word "shake" the following, "frequently for at least 15 min."

(3) The last sentence of the tentative qualitative test for gelatin (p. 223, 26) was deleted, and the method was amended by the addition of the following explanatory note:

In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize precipitates produced by picric acid when added to the Hg(NO<sub>3</sub>)<sub>2</sub> filtrates from these materials in the absence of gelatin. (Such samples, with or without rennet and entirely free from gelatin give, on standing, distinct precipitates when treated as above outlined. In every case, however, these precipitates differ in character from that which picric acid produces with gelatin. The gelatin-picric acid precipitate is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to the sides and bottom of the container from which it is rinsed with difficulty. Precipitates produced by picric acid in the absence of gelatin are flocculent, separate readily, leaving the serum practically clear, do not adhere to the walls of the container, and are easily removed by rinsing with H<sub>2</sub>O. When gelatin is present in the sample, the gelatin-picric acid precipitate will remain in suspension long after the flocculent precipitate has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to the bottom and sides of the test vessel. If gelatin is present in relatively high concentration (1%) the gelatin-picric acid precipitate will be voluminous and will settle rather quickly.)

When examining cottage cheese, mix thoroly 5 g of the sample with 10 cc of H<sub>2</sub>O at 50-60° and add 5 cc of Hg(NO<sub>3</sub>)<sub>2</sub> soln. Shake, let stand 5 min., and filter thru a medium fast, retentive paper. To the filtrate add 5 cc more of the Hg(NO<sub>3</sub>)<sub>2</sub> soln and test as before, using the filtrate so obtained for the test.

(4) The method for the determination of added water in cream (p. 225, 32) was made official (final action).

(5) The method of the American Public Health Association for the determination of sediment in milk, A.P.H.A. Standard Methods of Milk Analysis, 6th ed., 1934, pp. 44-46, was adopted as tentative.

(6) The Hartmann-Hillig method, *This Journal*, 15, 643 (1932), for the determination of citric acid in milk was adopted as tentative.

(7) The method submitted by the associate referee for the determination of fat in dried milk, *This Journal*, 15, 75 (1932), was adopted as official (final action), and provision was made for making the two procedures given under "Preparation of Solution" alternative methods.

(8) The tentative method for the determination of lactic acid in dried milk, *This Journal*, 18, 78 (1935), was dropped.

(9) The official method for the determination of ash and salt in cheese (p. 239, 93) was dropped (final action).

(10) The official method for the determination of fat in cheese (p. 239, 97) was dropped (final action).

(11) The official method for the determination of moisture in cheese (Method I, p. 238, 91) was amended by the insertion of the words "and process cheese" after the word "cheese," second line (final action).

### XXIII. EGGS AND EGG PRODUCTS

(1) The title of section 14, XXIII, p. 248, was changed from "Acidity of Fat" to "Acidity of Ether Extract."

(2) The method for the determination of ammonia nitrogen, *This Journal*, 6, 7 (1922), was adopted as tentative for liquid eggs and was inserted under the heading "Detection of Decomposition," p. 248.

### XXIV. FISH AND OTHER MARINE PRODUCTS\*

#### XXV. FLAVORING EXTRACTS

No additions, deletions, or other changes.

#### XXVI. FRUITS AND FRUIT PRODUCTS

(1) The method for the determination of inactive malic acid published in *This Journal*, 16, 281 (1933) was adopted as tentative.

(2) Minor changes were made in the tentative method for the determination of tartaric acid (p. 271, 28), but these changes do not affect the underlying principles of the method.

#### XXVII. GRAIN AND STOCK FEEDS

(1) The following revision submitted by the associate referee for the determination of calcium oxide in mineral feeds was substituted for the present tentative method (p. 287, 36):

##### CALCIUM OXIDE IN MINERAL FEEDS—TENTATIVE

Weigh a 2 g portion of the finely ground sample into a silica or porcelain dish and ignite in a muffle to a carbon-free ash, but avoid fusing. Boil the residue in 40 cc

of HCl (1+3) and a few drops of  $\text{HNO}_3$ . Transfer to a 250 cc volumetric flask, cool, dilute to mark, and mix thoroly. Pipet 25 cc of the clear liquid into a beaker, dilute to about 100 cc, and add two drops of methyl red indicator. Add  $\text{NH}_4\text{OH}$  (1+1) dropwise to a pH of 5.6, as shown by the intermediate brownish color. If overstepped add with a dropper HCl (1+3) to a brownish point and 2 drops in excess. The color should now be pink (pH 3.0-4.4) instead of brown. Dilute to about 150 cc, bring to boiling, and add slowly with constant stirring 20-30 cc of a saturated (4.2%) soln of  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , which should also be hot. If the red color changes to brown or yellow, add HCl (1+3) dropwise until the color again changes to pink. Let stand overnight to allow precipitate to settle. Filter the supernatant liquid through quantitative filter paper on a Gooch crucible, or on 1G4 sintered glass crucible (A. H. Thomas 4142), and wash the precipitate thoroughly with  $\text{NH}_4\text{OH}$  (1+50). Place the filter paper or crucible with the precipitate in the original beaker, and add a mixture of 125 cc of  $\text{H}_2\text{O}$  and 5 cc of  $\text{H}_2\text{SO}_4$ . Heat to  $70^\circ$  or above and titrate with 0.1 N  $\text{KMnO}_4$  until the first slightly pink color is obtained. Presence of filter paper may cause the pink color to fade in a few seconds. Correct for the blank and calculate the percentage of CaO in the sample.

(2) The following modified alkaline titration method submitted by the associate referee was substituted for 38, p. 287, and adopted as a tentative method:

#### ALKALINE TITRATION METHOD

Place 10-20 g of the sample, ground to pass a 20-mesh sieve, into an 800 cc Kjeldahl flask, and add about 200 cc of  $\text{H}_2\text{O}$ . (The autolysis should be conducted with the apparatus completely connected for distillation.) Collect 150-160 cc of distillate in a soln of NaOH (0.5 g in 20 cc of  $\text{H}_2\text{O}$ ). It is preferable to dilute to a volume of 250 cc and titrate a 100 cc aliquot.

To 100 cc of the distillate add 8 cc of 6 N  $\text{NH}_4\text{OH}$  and 2 cc of a 5% soln of KI and titrate with 0.02N  $\text{AgNO}_3$ , using a micro-buret. The end point is a faint but permanent turbidity, which may be easily recognized, especially against a black background. 1 cc of 0.02 N  $\text{AgNO}_3$  = 1.08 mg. of HCN.

(3) The following qualitative test described by the associate referee for detection of cyanogenetic glucosides was adopted as tentative:

#### CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS

##### *Qualitative Test*

Prepare sodium picrate paper by dipping strips into a 1% soln of picric acid and drying, then dipping into a 10% soln of  $\text{Na}_2\text{CO}_3$  and drying. Preserve these papers in a stoppered bottle. Finely chop a small quantity of plant material and place in a test tube. Insert a piece of the moist sodium picrate paper in the tube, taking care that it does not come in contact with the material. Add a few drops of  $\text{CHCl}_3$  and stopper the tube tightly. The sodium picrate paper gradually turns orange, then brick red if the plant tissue contains cyanogenetic glucosides. The test is delicate, and the rapidity of the change in color depends upon the amount of free hydrocyanic acid present. This test works well with fresh plant materials, but in the case of relatively dry substances, particularly the seeds of various plants, the material should be ground and moistened with  $\text{H}_2\text{O}$  and allowed to hydrolyze in a stoppered test tube containing sodium picrate paper. If necessary, a small amount of emulsin may be added.

(4) The qualitative tests for proteins described last year, *This Journal*, 18, 81 (1935), were adopted as official (first action).

(5) The method for the determination of salt (p. 278, 9) was adopted as official (final action).

#### XXVIII. MEATS AND MEAT PRODUCTS

No additions, deletions, or other changes.

#### XXIX. METALS IN FOODS

(1) The amendments to the Gutzeit method for the determination of arsenic (p. 306) suggested by the referee were adopted as official (first action).

(2) The precautionary note suggested by the referee was added to the tentative bromate method for the determination of arsenic, *This Journal*, 16, 75 (1933); 17, 70 (1934).

(3) The dithizone titrametric method for the determination of mercury, *This Journal*, 18, 640 (1935), was adopted as tentative.

(4) The colorimetric dithizone method for the determination of lead submitted by the referee was adopted as tentative (see p. 130).

(5) The electrolytic method for the determination of lead, *This Journal*, 17, 108 (1934); 18, 315 (1935), was adopted as tentative.

#### XXX. NUTS AND NUT PRODUCTS

The methods published in *This Journal*, 18, 419 (1935) were adopted as tentative.

#### XXXI. OILS, FATS, AND WAXES

(1) The following method for the identification of permitted colors was adopted as tentative:

##### COLORING MATTERS

Into each of four 500 cc separators measure out four 100 cc portions of the oil and dilute each funnel with 100 cc of low-boiling gasoline. Extract two or three times with 50 cc portions of 2 N  $\text{Na}_2\text{CO}_3$ , passing same successively through each funnel. If a yellow or pink color is obtained, test for Sudan G., annatto, or turmeric. Then extract the oil soln successively with three 50 cc portions of a mixture consisting of HCl and glacial acetic acid (1+5). A pink or red lower layer indicates aniline yellow (7) [15], butter yellow (16) [19], yellow AB ( ) [21], or yellow OB ( ) [61]. For detailed separation see XXI.

(2) The specifications for titer thermometer (p. 317, 14) were dropped (final action under suspension of the rules).

(3) The following specifications for titer thermometer were adopted as official (first action).

#### SPECIFICATIONS FOR TITER TEST THERMOMETERS

##### EXPLANATORY STATEMENT

The original specification for the titer test thermometer is about twenty years old and on account of certain arbitrary limits in the specifications has always been a difficult and expensive thermometer to manufacture. It appears, furthermore, that it was originally designed to be read to 1/10 or 1/5 of a division, that is, to 0.01°

or  $0.02^{\circ}$ , whereas, in practice, it is read to the nearest division, or perhaps occasionally to  $1/2$  division. The original specifications were difficult principally because it was desirable to keep the thermometer as short as possible, and this resulted in crowding the division marks so close together that reading is not easy. A slightly shorter, much more easily readable thermometer is obtained by subdividing the scale to  $0.2^{\circ}$  C. with a scale sufficiently open to make reading to  $1/2$  division,  $0.1^{\circ}$ , easy. This thermometer has been designed so as to cause no undue difficulties in manufacture, and at the same time to meet fully the requirements for accuracy in the titer test.

#### SPECIFICATIONS

*Type*.—Etched stem, glass.

*Liquid*.—Mercury.

*Range and subdivision*.—Minus 2 to  $62^{\circ}$  in  $0.2^{\circ}$ , with expansion chamber at top.

*Total length*.—350–360 mm.

*Stem*.—Plain front, enamel back, suitable thermometer tubing. Diameter, 6–7 mm.

*Bulb*.—Corning normal or equally suitable thermometric glass. Diameter not less than 5.5 mm but not greater than that of the stem. Length, 20–30 mm.

*Distance to  $-2^{\circ}$  mark from bottom of bulb*.—45–60 mm.

*Distance to  $62^{\circ}$  mark from top of thermometer*.—20–50 mm.

*Length of unchanged capillary*.—Between top of bulb and the first graduation mark, 13 mm, and between the last graduation mark and the expansion chamber at the top, 10 mm.

*Top finish*.—Glass ring or knob.

*Filling above mercury*.—Nitrogen or other suitable gas.

*Graduation*.—All lines, figures, and letters to be clear cut and distinct. Each degree mark to be longer than the remaining lines. Graduations to be numbered at every  $2^{\circ}$  mark.

*Immersion*.—Total.

*Special marking, A.O.A.C. titer test*.—A serial number, and the manufacturer's name or trade mark shall be etched upon the stem. The marking " $0.2^{\circ}$ C." shall be marked on the front of the stem above the scale.

*Scale error*.—The error at any point on the scale, when the thermometer is standardized at total immersion shall not exceed  $0.2^{\circ}$  C.

*Case*.—The thermometer shall be supplied in a suitable case on which shall appear the marking: *A.O.A.C. Titer Test*,  $-2^{\circ}$  to  $62^{\circ}$  C. in  $0.2^{\circ}$ .

**NOTE:** For the purpose of interpreting these specifications the following definitions apply:

The total length is the over-all length of the finished instrument.

The diameter is that measured with a ring gage.

The length of the bulb is the distance from the bottom of the bulb to the beginning of the enamel backing.

The top of the thermometer is the top of the finished instrument.

(4) The following alternative procedures of Malfatti and of Stout and Schuette were substituted for Reagent (b), *Alcoholic potassium hydroxide soln*, in the saponification number method (p. 321, 21 (first action)):

(b) *Alcoholic potassium hydroxide soln*.—(1) Reflux 1.2 liters of 95% alcohol for 30 min. in a distilling flask with 10 g of KOH and 6 g of granulated Al (or foil). Distill, and collect 1 liter after discarding the first 50 cc. Dissolve 40 g of high-grade KOH in this liter of alcohol. Keep soln in a glass-stoppered bottle. Or (2) Crush 40 g of high-grade KOH in a 7 or 8 in. mortar. Add 45 g of granulated CaO and grind

mixture to a powder. From a liter of 95% alcohol add 100 cc to the mortar and transfer to a flask, rinsing the mortar with several more portions. Add the remainder of the alcohol to the flask, shake the mixture for at least 5 min. and then invert a beaker over the neck of the flask. Repeat the shaking several times during the day. Next morning filter the soln into a clean, dry, glass-stoppered bottle.

(5) Method I, tentative for the detection of foreign fats containing tristearin in lard (p. 331, 46), was dropped.

(6) Method II for the same determination (p. 332, 47) was revised to read as follows:

#### DETECTION OF FOREIGN FATS CONTAINING TRISTEARIN IN LARD—TENTATIVE

Weigh 5 g of the melted and filtered lard into a glass-stoppered cylinder and add 20 cc of warm acetone. Mix well, taking care that the soln is clear and has a temp. above 30°. Let stand at a constant temp. of 30° for 16–18 hours. A fine mass of crystals occupying a volume of not more than 3 cc should then be found at the bottom of the cylinder. Should the volume of crystals materially exceed 3 cc, take a smaller quantity of lard (3–4 g) for a new test. Should no crystals be deposited, as may be the case with soft or oily lard, absence of tristearin is indicated. Decant the supernatant acetone soln from the crystallized glycerides. Add warm (30–35°) acetone in three portions of 5 cc each from a small wash bottle, taking care not to break up the deposit in washing, and decant the first two portions. Actively agitate the third portion in the cylinder, and by a quick movement transfer the crystals to a small filter paper. Using the wash bottle, wash the crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out the paper and its contents, breaking up any large lumps and allow to dry in air at room temp. Thoroughly comminute the mass and take the melting point of the crystals in a closed 1 mm tube, using an apparatus similar to that indicated under 14. Heat the H<sub>2</sub>O in the beaker rapidly to about 55° and maintain this temp. until the thermometer carrying the melting point tube registers 50°, then heat again and raise the temp. of the outer bath rather quickly to 67°. Remove the burner. The melting point is reached when the fused substance becomes perfectly clear and transparent. When the melting point of the glycerides obtained by this method is below 63.6° the presence of beef fat or other fat containing tristearin should be suspected, and a melting point of 63.2° or lower is evidence that the sample is not pure lard. It is advisable to carry out the method with a control sample of pure lard.

The conclusion indicated by the melting point may be confirmed by taking the melting point of the fatty acids prepared from the glycerides. After determining the melting point, transfer the crystallized glycerides to a 50 cc beaker, add 25 cc of approximately 0.5 *N* alcoholic KOH, and heat on a steam bath until saponification is complete. Pour the soln into a separatory funnel containing 200 cc of H<sub>2</sub>O, acidify, add 75 cc of ether, shake, and let stand. Draw off the aqueous acid layer and wash the ether soln at least 3 times with H<sub>2</sub>O. Transfer the ether soln to a clean dry 50 cc beaker, volatilize ether on the steam bath, and finally dry the acids at 100°. After about 2 hours, determine the melting point.

Conclusions may be confirmed further by precise determinations of the mean molecular weight of the separated fatty acids. Use a 0.5–0.2 *N* standard KOH soln and dissolve them in colorless, redistilled alcohol, which has been carefully neutralized immediately before use. If the sample is pure lard, the mean molecular weight of the fatty acids should correspond closely to that of the fatty acids of a palmitodistearin, 274.67. If the sample is impure, the mean molecular weight should tend to approach that of the fatty acids from tristearin, 284.



**XXXII. PRESERVATIVES**

No additions, deletions, or other changes.

**XXXIII. SPICES AND OTHER CONDIMENTS**

(1) The following method for the evaluation of ginger<sup>1</sup> was adopted as tentative:

Place 50 g of ground ginger in a Soxhlet extractor and extract completely, using ether as the solvent (approximately 4 hours). Transfer the extract to a 300 cc flask and evaporate off the ether on the steam bath until the solvent is no longer detected. Add 50 cc of H<sub>2</sub>O to the residue and determine the yield of volatile oil (using trap for oils lighter than H<sub>2</sub>O) and determine specific gravity, optical rotation, refractive index, acid and ester numbers as directed under XXXIII.

Transfer the residue in the flask to a separatory funnel and extract the resin with ether. Transfer to a tared beaker, evaporate the ether on a steam bath, and dry to constant weight in a vacuum desiccator.

(2) The method for the determination of total sulfur in mustard, (p. 351, 16) was dropped (final action under suspension of the rules).

**XXXIV. SUGARS AND SUGAR PRODUCTS**

(1) The table of conversion factors for different saccharimeter scales, *This Journal*, 18, 162 (1935), was adopted for inclusion in the 4th edition of *Methods of Analysis*.

(2) The action taken in 1931, *This Journal*, 15, 78 (1932), deleting the method for the determination of invert sugar in honey, p. 390, 97, 98, was rescinded, and the method then adopted was dropped, pending further study.

**XXXV. VEGETABLES AND VEGETABLE PRODUCTS**

(1) Certain editorial changes suggested by the referee were made in the tentative methods for the determination of total solids and insoluble solids in tomato products (p. 398, 13, 14) to clarify the text.

(2) The following method for the determination of specific gravity in tomato products was adopted as tentative:

Determine the sp. gr. at 20/20°, using a National Canners Association sp. gr. bottle.<sup>2</sup> Clean and calibrate the bottle at 20° as directed in XVII, 24, but since the bottle is not provided with a cap, strike off excess H<sub>2</sub>O with a straight edge, wipe the bottle dry, and weigh immediately. Cool the sample to 16–18°, fill the flask with the pulp, and place it in a centrifuge with a suitable counterpoise in the other receptacle. Whirl for 1 min. at a speed of about 1000 r.p.m. Add sufficient pulp to fill the flask to the top and whirl the centrifuge again. Remove flask and take the temp. of the pulp, inserting the thermometer so that no air is introduced. When the temp. is just 20°, remove thermometer, add sufficient pulp at the same temp. to have the flask slightly over-full, and strike off even with a straight edge. Clean the outside of the flask and weigh at once to the nearest 0.01 g. Sp. gr. = weight of pulp in the flask ÷ the weight of H<sub>2</sub>O at 20° that the flask holds.

<sup>1</sup> *J. Am. Pharm. Assoc.* 17, 630 (1928).

<sup>2</sup> N.C.A. Bull. 21L, p. 136.

(3) The methods for the micro analysis of tomato pulp, etc. (p. 400, 26-29, inclusive) were adopted as official (final action under suspension of the rules).

### XXXVI. VITAMINS\*

### XXXVII. WATERS, BRINE AND SALT

(1) The following method for the determination of fluorides was adopted as tentative:

#### FLUORIDES<sup>1</sup>—TENTATIVE

##### REAGENTS

(a) *Standard fluoride soln.*—1 cc = 0.02 mg of F. Prepare by diluting a stock soln containing 1 g of F per liter. NaF may be weighed out directly (2.22 g to 1 liter).

(b) *Titanium soln.*—To 2 cc of 20%  $\text{TiCl}_3$ , add 40 cc of  $\text{HNO}_3$  (1+1) and make to 1 liter with  $\text{H}_2\text{O}$ .

(c) *Hydrogen peroxide soln.*—Dilute 10 cc of 30%  $\text{H}_2\text{O}_2$  to 100 cc with  $\text{H}_2\text{O}$ .

(d) *Copper nitrate soln.*—Dissolve 5 g of  $\text{Cu}(\text{NO}_3)_2$  in 100 cc of  $\text{H}_2\text{O}$ .

Test the  $\text{H}_2\text{SO}_4$  for presence of fluorine. A straight Willard and Winter distillation<sup>2</sup> will suffice. Collect 175 cc distillate and make to 200 cc volume.

##### APPARATUS

(a) *Claissen flask.*—Capacity 125 cc.

(b) *Nessler tubes.*—At least 7 long-form 50 cc tubes, and more if possible. Tubes with fused bottoms of optical glass are desirable but not absolutely necessary. (Fisher Scientific Company's "Double Plane" tubes are recommended.) Match the tubes for length and test for optical similarity by filling to the mark with a soln corresponding to the "0.04" standard described later. Reject all tubes showing detectable differences in shade or intensity.

(c) *pH comparator.*—Use any instrument that will show that the pH of a colored soln equals  $1.50 \pm 0.02$  pH. A special set made by the LaMotte Company has standards of 1.40, 1.45, 1.50, 1.55, and 1.60 pH units with metacresol purple indicator.

##### PREPARATION OF SAMPLE

Make a preliminary examination to ascertain the approximate quantity of F present by comparing in Nessler tubes 10 cc of the clear sample (filtered if necessary) with known F standards containing not more than 0.05 mg of F (waters containing sulfates, phosphates, Al salts, and other interfering substances vitiate the colorimetric comparison and give erroneous results).

According to the approximate F content, take a suitable quantity of  $\text{H}_2\text{O}$ , make neutral to phenolphthalein indicator with 2.5% NaOH soln, and evaporate in porcelain to 5 cc. Transfer to the Claissen flask, using not more than 25 cc of  $\text{H}_2\text{O}$ , and proceed as directed in XV.

##### ISOLATION

Put into the Claissen flask 20-30 glass beads and place the flask on an asbestos mat with an opening large enough to expose about one-fourth of the flask to the flame. Close the straight neck of the flask with a two-holed rubber stopper through which pass a thermometer and a small separatory funnel, the outlet end of which is constricted to a diameter of about 2 mm. Have the thermometer and the funnel extend almost to the bottom of the flask. Close the other neck of the flask with a solid rubber stopper. Connect the flask with a water condenser, add 7 cc of  $\text{H}_2\text{SO}_4$

<sup>1</sup> *This Journal*, 16, 612 (1933); 17, 204 (1934).

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).

through the separatory funnel, mix, and distil. Keep the temp. at  $135^{\circ} \pm 3^{\circ}$  during the distillation, regulating it by addition of  $H_2O$  from the separatory funnel. Collect 200 cc of distillate in a volumetric flask.

#### DETERMINATION

(a) *Color Standards*.—To each of 6 Nessler tubes add 1.0 cc  $HNO_3$  (1+9) and 1.00 cc (accurately measured) of the  $TiCl_3$  soln, then 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 cc of the standard F soln. Add 30 cc of  $H_2O$  and 2 cc of the peroxide soln, make to mark, and mix by inverting the tubes at least 5 times. Test the pH of one of the series and replace. This gives a series of standards reading 0.00 to 0.05 mg of F. If the tubes are kept stoppered and protected from intense sunlight, they will keep constant for 24 hours.

If trouble is experienced in matching the yellow colors add, before making to volume, 2.0–2.5 cc (amount varying with the analyst's color preference) of the  $Cu(NO_3)_2$  soln (not sulfate, which introduces an interference). The resultant color varies from yellowish green (no F) through green to bluish green.

(b) *Sample Tubes*.—Mix distillate, and filter, if necessary, on a good grade quantitative filter. (Certain qualitative filters, particularly when old, have been found to impart appreciable amounts of yellow color to the filtrate.)

Prepare one or more sample tubes as directed under (a) substituting for the standard F soln a suitable aliquot of the distillate. For greatest accuracy use an aliquot that contains as near as possible (*but not exceeding*) 0.05 mg of F.

*Note 1*.—The quantities of  $HNO_3$  mentioned are approximate. Each analyst is expected to determine the accurate figures applicable to his own supply. The pH of the sample and the standards must be the same (within  $\pm 0.02$ ), and both should be pH 1.5 or slightly lower.

*Note 2*.—The limits of accuracy of the other reagents are:

|                        | cc | cc         |
|------------------------|----|------------|
| Titanium soln.....     | 1  | $\pm 0.01$ |
| Hydrogen peroxide..... | 2  | $\pm 0.02$ |
| Copper nitrate.....    | 2  | $\pm 0.02$ |

#### (c) *Color comparisons*.

(1) *Standard series method*.—When the proper amount of  $HNO_3$  (1+9) has been found giving the same pH in the sample tube as that in the color standards, make up a new sample tube and compare with the standards. Uniform, moderate illumination, a minimum of color interference by shadows or surrounding objects, and a minimum of eye fatigue are necessary for uniform results. A three-compartment box comparator can easily be made and is a handy instrument.

(2) *Duplication method*.—Prepare a sample and one standard tube as described in (a), except that to the standard tube no F is added, and it is made to a volume of approximately 48 cc. Titrate into the standard tube a F soln containing 0.2 mg of F per cc, until colors of sample and standard match, mixing *both* tubes by inverting 5 times after each addition. After taking the final buret reading, add 0.25 cc of F soln (0.005 mg F). A distinct color change should be visible.

Regardless of whether the standard series or the duplication method of comparison is used, the amount of F present in the aliquot taken *must not exceed* 0.05 mg. Above 0.05 mg of F the change in color per unit of F is less than below this limit. Hence, wherever a sample aliquot is found to contain more than 0.05 mg of F, *it is absolutely necessary* to repeat this determination on another aliquot small enough to bring the F content of the tube within the 0.05 mg limit.

(2) Method I, official for the determination of arsenic (p. 420, 78 and 79) was dropped (final action under suspension of the rules).

**XXXVIII. RADIOACTIVITY OF FOODS AND DRUGS**

(1) The following gamma ray method for the determination of radioactivity was adopted as tentative:

*Gamma ray method***APPARATUS**

A cylindrical zinc chamber (1) of about 1000 cc capacity, which is hermetically sealed. The axis of the cylinder is vertical. On the inside is the Wulf two-fiber system (3), which is fastened to an amber insulator, and which can be charged with the aid of a charging-rod (4). The rate of movement of the fibers is determined by means of a microscope (2).

**PREPARATION OF SAMPLE**

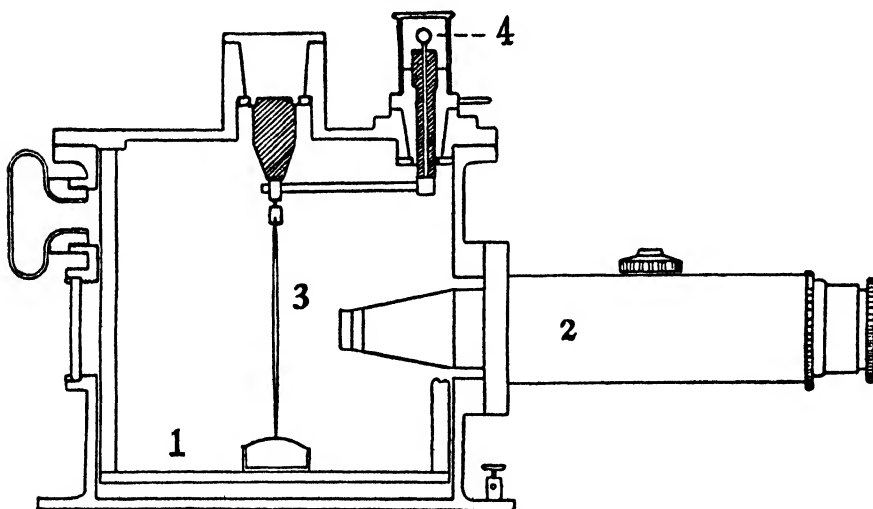
Use the whole sample or one or more subdivisions, depending upon the content of radioactivity, but do not open the individual containers. Seal any loose material in a suitable container such as a test tube.

**STANDARDS**

Use known quantities of radium measured by the U. S. Bureau of Standards.

**STANDARDIZATION OF ELECTROSCOPE**

(a) *Natural leak.*—Charge the electroscop through the charging rod by means of a charging device, 2 (g), to bring one of the fibers to a suitable point on the properly illuminated microscope scale after 4 is grounded—for example, at the



**GAMMA RAY ELECTROSCOPE**

50 division mark or above. As the natural leak of the electroscop in a room free from radium is very small, use a radium standard to adjust the fiber approximately to the desired division mark. Remove the standard from the room and record the time when the fiber crosses the exact division mark. Allow the electroscop to remain charged overnight. Again record the time when the fiber crosses an exact division mark. Calculate the rate of travel of the fiber in seconds per division and designate the figure obtained as the natural leak (R) of the electroscop for the particular determination:

(b) *Constant*.—Place a suitable radium standard containing 10–1000 micrograms of radium at an exact measured distance from the center of the electroscope. Charge the electroscope and record the average time, measured by a stop-watch, for at least 6 trials of the fiber to travel over that part of the scale used in obtaining the natural leak. Calculate the rate of travel in seconds per division and the corrected time (T) due to radium alone by the following formula:

(1)  $T = AR/R - A$ , in which A = observed time and R = natural leak.

Then calculate the constant (K) by the following formula:

(2)  $K = ST/(D)^2$ , in which S = micrograms of radium in the standard; T = corrected time found in (1); and D = distance between the center of the electroscope and the standard.

To obtain a reliable average figure for this constant, calculate K, placing the radium standard at different distances from the center of the electroscope. Use several different standards of known radium content.

#### DETERMINATION

Place the sample at a suitable distance from the center of the electroscope. Charge the electroscope as directed above, using if convenient a radium standard to adjust the fiber. Record the average time taken by the fiber to travel between exact division marks over all or a major portion of that part of the scale used for the standardization. If the sample contains sufficient radioactivity to permit, take average readings when it is placed at different distances from the center of the electroscope; if it contains only a relatively small quantity of radioactivity, fasten it with rubber bands to the circumference of the electroscope so as to obtain the maximum ionization. Calculate the micrograms of radium (S') or its equivalent in terms of radium by the following formula:

(3)  $S' = K(D')^2/T'$ , in which K = constant of electroscope; D' = exact distance between the center of the electroscope and the center of the sample; and T' = corrected time in seconds per division due to the radioactivity only in the sample.

(2) The radon method (official, first action), *This Journal*, 14, 85 (1931), was adopted as official (final action).

(3) The title of this chapter was changed to read "Radioactivity."

(4) The emanation method, p. 433, 4 (b) (2), was modified to provide for the presence or absence of appreciable quantities of barium sulfate.

#### XXXIX. DRUGS

(1) The following microchemical methods for the identification of theobromine and theophylline were adopted as tentative:

##### THEOBROMINE AND THEOPHYLLINE

##### REAGENTS

(a) *Ammoniacal silver nitrate soln.*—Mix 5 cc of 2%  $\text{AgNO}_3$  with 5 cc of 10%  $\text{NH}_4\text{OH}$ .

(b) *Kraut's*.—Dissolve 4 g of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  in 10 cc of  $\text{HNO}_3$  (1+1). Dissolve 13.6 g of KI in 25 cc of  $\text{H}_2\text{O}$ . Mix the solns and dilute to 50 cc. Use freshly prepared reagent only.

(c) *Mercuric chloride soln.*—Dissolve 5 g of  $\text{HgCl}_2$  in 100 cc of  $\text{H}_2\text{O}$ .

(d) *Controls*.—Prepare a soln of the pure alkaloid in the concentration directed.

##### IDENTIFICATION

Place a drop of the alkaloidal soln on a clean, glass slide; add a drop of reagent by means of a clean, glass rod; and, without stirring or covering, examine under the

microscope, using low power. A magnification of 100-150 is suitable. Note the kind of crystals formed and compare their characteristics with the descriptions given and also with a control.

#### CHARACTERISTICS OF MICROCHEMICAL TESTS FOR ALKALOIDS

| ALKALOID     | REAGENT                    | DESCRIPTION OF CRYSTALS  |
|--------------|----------------------------|--|
| Theobromine  | Kraut's (freshly prepared) | In hydrochloric acid (1+3) tufts of brown, radiating needles form readily in 1:200 soln  |
| Theophylline | Ammoniacal silver nitrate  | Gelatinous at first; dense spheres of dark radiating needles form in 1:200 soln. Crystals form more readily if prepared in test tube and then transferred to a glass slide |

(2) The following microchemical methods for the identification of acetanilid, acetphenetidin, and neocinchophen were adopted as tentative:

#### ACETANILID, ACETPHENETIDIN AND NEOCINCOPHEN

##### REAGENTS

- (a) *Platinic chloride soln.*—Dissolve 1 g of  $H_2PtCl_6$  in 20 cc of  $H_2O$ .  
 (b) *Potassium thiocyanate soln.*—Dissolve 5 g of KSCN in 100 cc of  $H_2O$ .  
 (c) *Nitric acid soln.*—Mix one volume of  $HNO_3$  with one volume of  $H_2O$ .  
 (d) *Phosphotungstic acid soln.*—Dissolve 5 g of  $P_2O_5$ , 24  $WO_3$ ,  $xH_2O$  in 100 cc of  $H_2O$ .  
 (e) *Bromide-bromate soln.*—Dissolve 0.3 g of  $KBrO_3$  and 5 g of KBr in  $H_2O$  and dilute to 100 cc.  
 (f) *Wagner's.*—Dissolve 1 g of I and 5 g of KI in 5 cc of  $H_2O$  and dilute to 100 cc.

##### PREPARATION OF SAMPLE

Separate the compound for microchemical testing in pure form by the use of suitable solvents. Prepare a soln with the aid of acid, alkali, or  $H_2O$  in the concentration specified for the individual synthetics.

*Controls.*—For comparison, prepare a soln of the known synthetics in the concentration specified for each.

##### IDENTIFICATION

To a drop of the soln of the compound or to about 1 mg of the powder on a glass slide, add a drop of the specified reagent. Without stirring or covering, examine under the microscope. A magnification of about 100 is suitable. Note the characteristics of the crystals formed and compare with description and control.

| SYNTHETIC      | SOLUTION                            | CONCENTRATION OF SYNTHETIC | REAGENT              | DESCRIPTION OF TESTS AND CRYSTALS  |
|----------------|-------------------------------------|----------------------------|----------------------|--|
| Acetanilid     | 10% HCl                             | 1:100                      | Phosphotungstic acid | Rosettes of prisms   |
| Acetanilid     | 10% HCl                             | 1:100                      | Bromide-bromate      | Small prisms   |
| Acetphenetidin | About 1 mg of the powdered material |                            | Nitric acid          | After adding a drop of nitric acid let stand for a few seconds, then add a drop of $H_2O$ . Bright yellow, curving branched crystals |

|                     |         |                   |                            |   |
|---------------------|---------|-------------------|----------------------------|---|
| Acetphen-<br>etidin | 10% HCl | Saturated<br>soln | Wagner's                   | Large irregular plates  |
| Neocincho-<br>phen  | 10% HCl | Saturated<br>soln | Potassium thiocy-<br>anate | Rosettes of needles.<br>(Gentle agitation by<br>tipping the slide back<br>and forth hastens<br>crystallization) |
| Neocincho-<br>phen  | 10% HCl | Saturated<br>soln | Platinum chloride          | Needles in clusters   |

(3) The following method for the determination of santonin in *santonica* was adapted as a tentative method:

#### SANTONIN IN SANTONICA (LEVANT WORM SEED)

Extract 3 g of the ground sample with benzene in a Soxhlet apparatus or an automatic percolator, *This Journal*, 15, 629 (1932), for 3 hours. Wash the extract into a separator with a little benzene, add more benzene if necessary to make a total volume of approximately 100 cc, and shake vigorously for 5 min. with 35 cc of 8%  $\text{Na}_2\text{CO}_3$  soln. Allow the mixture to separate completely and transfer the aqueous layer to a second separator. Wash the benzene once with 10 cc of  $\text{H}_2\text{O}$  and add the washing to the second separator. Shake the combined aqueous extracts with 10 cc of benzene, discard the aqueous layer, wash the benzene with 5 cc of  $\text{H}_2\text{O}$ , and combine with the benzene in the first separator. Filter the benzene soln through cotton and evaporate the filtrate to dryness. Warm the residue with 5 cc of alcohol until the mass is disintegrated, and add 60 cc of saturated aqueous soln of  $\text{Ba}(\text{OH})_2$ , while stirring. Heat the mixture to boiling, place on the steam bath for 10 min., filter into a separator, and wash the filter and beaker with two 10 cc portions of hot  $\text{Ba}(\text{OH})_2$  soln. Add 6 cc of  $\text{HCl}$  (2+1) to the filtrate, cool, and extract with 25, 15, 10, 10, and 5 cc portions of chloroform, filtering through a pledget of cotton in the stem of the funnel, and evaporate the filtrate to dryness. Dissolve the residue in 25 cc of alcohol by warming, mix the soln with 50 cc of dinitrophenylhydrazine sulfate soln, *This Journal*, 18, 87, (1935), and proceed as directed in the assay of santonin in mixtures, beginning with the words "allow to stand for 48 hours."

(4) The following method for the determination of the swelling factor of psyllium was adopted as tentative:

#### SWELLING FACTOR OF PSYLLIUM APPARATUS

Select the required number of 50 cc graduated cylinders and provide them with one-holed rubber stoppers (usually Nos. 3 or 4). Through the stoppers insert glass stirring rods of such diameters that they will slide easily in the holes and of such lengths that any material in the bottom of the cylinders may be stirred conveniently and thoroly.

#### DETERMINATION

Place 1 g of psyllium (seeds) in a cylinder, add  $\text{H}_2\text{O}$  to the 20 cc mark, and stir well. Draw the rod out of the liquid after each stirring by sliding through the stopper. Place the cylinder and its contents in a refrigerator or a cool place ( $5-10^\circ$ ) for 24 hours, stirring the contents at frequent intervals. Remove the cylinder, stir, and allow the contents to settle for 1 hour at room temp. (or until no further change is observed in the total volume occupied by the drug). This final reading (to one

decimal place) is taken as the swelling factor of the drug. (Note: In the case of *Lallemantia royleana*, add H<sub>2</sub>O to the 50 cc mark in the cylinder.)

(5) The following method for the determination of theobromine in theobromine calcium was adopted as tentative:

#### THEOBROMINE IN THEOBROMINE CALCIUM

Dry about 0.5 g of the material at 110° to constant weight. Weigh 0.2 g of the dried substance into a glass-stoppered 100 cc volumetric flask, add 2 cc of glacial acetic acid, and warm on the steam bath. Add 10 cc of boiling H<sub>2</sub>O and shake until solution has taken place, adding more boiling H<sub>2</sub>O if necessary. Cool the soln to room temp. (The soln should be clear or nearly so.)

Add 50 cc of 0.1 *N* iodine, 20 cc of saturated salt soln and 2 cc of HCl. Shake well and make to volume with H<sub>2</sub>O. Shake again and allow to stand overnight. Filter, discarding the first 10 cc of the filtrate. Titrate 50 cc of the filtrate with 0.1 *N* Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, using starch soln as indicator. 1 cc of 0.1 *N* I = 0.0045 g of theobromine.

(6) The methods for the titration of quinine (XXXIX, 11 and 107) were amended to substitute bromocresol purple for methyl red as indicator, and the following method for the preparation of bromocresol purple solution was adopted as tentative:

*Bromocresol purple soln.*—Triturate 0.100 g of bromocresol purple in an agate mortar with 9 cc of 0.02 *N* NaOH. After solution dilute with distilled H<sub>2</sub>O to 200 cc, and filter if necessary. The soln should be deep orange to red in color. If it is purple, the addition of not more than 0.50 cc of 0.02 *N* acid should make it red. If it is yellow, the addition of not more than 0.50 cc of 0.02 *N* alkali should produce the red color.

(7) The method for separating acetphenetidin and caffeine, XXXIX, 17 (a), was amended by inserting the expression, "The diluting and heating process must be repeated until acetic acid can no longer be detected in the vapors" after the words "liquid amounts to 8–10 cc."

(8) The directions for the determination of the melting point of acetylsalicylic acid (XXXIX, 20) were deleted, and the expression, "Use the U. S. P. method" substituted therefor.

(9) The qualitative tests for ephedrine (XXXIX, 48) were deleted to avoid duplication in U.S.P. XI.

(10) The method for the assay of mercuric iodide in tablets (XXXIX, 66, 67) was deleted to avoid duplication with N.F. VI.

(11) The microchemical test for diacetylmorphine, XXXIX, 76 (b), was deleted to avoid duplication (33, 34 and 35).

(12) The method for the assay of pepsin (XXXIX, 78, 79, 80), using ricin as reagent, was deleted.

(13) The iodine method for phenolphthalein in plain tablets (XXXIX 81, 82, 83) was deleted to avoid duplication in N.F. VI.

(14) The title "Procaine Hydrochloride (Novocaine)" (XXXIX, 100) was changed to "Procaine."

(15) Three identification tests for procaine hydrochloride, 100 (d), (e), and (f), were deleted to avoid duplication in U.S.P. XI.



(16) Method I for the assay of procaine hydrochloride (XXXIX, 102) was amended by the insertion at the beginning of the text of this expression: "This method determines as procaine any *p*-amino-benzoic acid formed from the decomposition of procaine."

(17) Method II for the assay of procaine hydrochloride (XXXIX, 103) was amended by the insertion at the beginning of the text of this expression: "This method determines only undecomposed procaine."

(18) The official method for the assay of strychnine in tablets (XXXIX, 106) was amended so that the paragraph beginning with the words, "Add 2-3 cc of neutral alcohol," reads as follows:

Add 2-5 cc of neutral alcohol. Cover the beaker and warm on a steam bath to dissolve the residue. If necessary, add just enough additional alcohol to complete the soln. Add 2 drops of methyl red indicator, 38(e), and titrate with 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to a faint pink color. If more than 2 cc of alcohol was used, evaporate the excess, cool, dilute with 50 cc of recently boiled H<sub>2</sub>O, and continue the titration with the 0.02 H<sub>2</sub>SO<sub>4</sub>. If preferred, add an excess of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> to the alcoholic solution of the alkaloids, evaporate the alcohol, if necessary, as directed above, and titrate the excess acid with 0.02 *N* NaOH.

1 cc of 0.02 *N* H<sub>2</sub>SO<sub>4</sub> = 0.006686 g of C<sub>21</sub>H<sub>23</sub>O<sub>2</sub>N<sub>2</sub>; 0.008568 g of (C<sub>21</sub>H<sub>23</sub>N<sub>2</sub>O<sub>2</sub>)<sub>2</sub> · H<sub>2</sub>SO<sub>4</sub> · 5H<sub>2</sub>O; or 0.007946 g of C<sub>21</sub>H<sub>23</sub>O<sub>2</sub>N<sub>2</sub> · HNO<sub>3</sub>.

(19) The tentative method for the assay of arsenic in iron-arsenic tablets (XXXIX, 117) was made official, final action, under suspension of the rules.

(20) The method for the determination of arsenic in arsphenamine and neoarsphenamine (XXXIX, 121) was deleted to avoid duplication in U.S.P. XI.

(21) The following alternative method for the determination of barbital and phenobarbital was adopted as tentative:

Dissolve the residue obtained, 140, in 10 cc of alcohol, add 20 cc of a saturated, aqueous soln of Ba(OH)<sub>2</sub>, and stir well. Filter into a separator and wash the residue and filter with two or three 10 cc portions of the Ba(OH)<sub>2</sub> soln. Acidify the soln with 10% HCl and proceed as directed under 140, beginning with the words, "Extract 5 times."

(22) The method for barbital and phenobarbital, XXXIX, 138 (b), was amended to change the solvent so as to read "Mix 20 cc of ether and 80 cc of chloroform."

(23) The methods for the detection of excess fatty acids and castor oil in chaulmoogra oil (XXXIX, 143, 144, p. 484) were deleted to avoid duplication with U.S.P. XI.

(24) The following methods (official, first action) were made official, final action: Ephedra (XXXIX, 43), Camphor, *Ibid.*, 55, Pyramidon, *Ibid.*, 98, 99, Arsenic in Iron Methylarsenate, *Ibid.*, 120, Mercurous Chloride (Calomel) in Tablets, *Ibid.*, 128, and the Cat-eye Method for the Assay of Mydriatics and Myotics, *Ibid.*, 150, 152, 153.

(25) The following tentative methods were adopted as official (first action): Acetanilid and caffeine, 5; acetanilid, caffeine, and codeine, 8; acetanilid, caffeine, and quinine, 10; acetanilid, caffeine, quinine, and morphine, 12; acetanilid and sodium salicylate, 14; antipyrin and caffeine, 31; pilocarpine, 37; emetine hydrochloride in tablets, 38; atropine in tablets, 39; ephedrine in inhalants, 44; ephedrine in tablets, 46; apomorphine in tablets, 72; menthol, 92; and thymol, 94.

#### **XL. BACTERIOLOGICAL METHODS\***

#### **XLI. MICROCHEMICAL METHODS\***

#### **XLII. TABLES**

The changes made in the tables are given under the chapters involved.

#### **DISINFECTANTS**

The U.S. Food and Drug Administration method (F.D.A. method) for determining the phenol coefficient (including both *Eberthella typhi* and *Staphylococcus aureus*), as published in U.S.D.A. Circ. 198, was adopted as a tentative method.

#### **LIGNIN**

The modified fuming hydrochloric acid method for the quantitative estimation of lignin submitted by the referee, *This Journal*, 15, 126 (1932), was adopted as tentative.

#### **STANDARD SOLUTIONS**

The following methods for the preparation and standardization of acid and alkali were adopted as tentative:

#### **STANDARD SOLUTIONS OF SODIUM HYDROXIDE**

##### **APPARATUS**

The buret and pipet used should be Bureau of Standards calibrated or should be calibrated by the analyst. Automatic burets should have all exits to the air protected from CO<sub>2</sub> contamination by suitable guard tubes containing soda-lime. All containers should be of alkali-resisting glass.

##### **REAGENTS**

(a) *Carbonate-free H<sub>2</sub>O*.—Prepare by one of the following methods: (1) Boil distilled H<sub>2</sub>O 20 min. Cool with soda-lime protection; (2) bubble air, freed from CO<sub>2</sub> by passing through a tower of soda-lime, through distilled H<sub>2</sub>O for 12 hours.

(b) *1 + 1 Alkali*.—To one part of NaOH (reagent quality containing less than 5% Na<sub>2</sub>CO<sub>3</sub>) in a flask add one part of distilled H<sub>2</sub>O and swirl until soln is complete. Close with rubber stopper. Set aside until Na<sub>2</sub>CO<sub>3</sub> has settled, leaving a perfectly clear liquid (about 10 days).

(c) *Potassium acid phthalate*.—U. S. Bureau of Standards Sample Standard for Acidimetry. Dry for 2 hours at 120°. Cool in a desiccator containing H<sub>2</sub>SO<sub>4</sub>.

(d) *Phenolphthalein indicator*.—1.0 g in 100 cc of 95% alcohol.

## PREPARATION OF STANDARD SOLUTION

The following table gives the approximate amount of 1+1 alkali necessary to make 10 liters of standard soln:

| <i>Approx. Normality</i> | <i>1+1 Alkali to be diluted to 10 liters</i><br><i>cc</i> |
|--------------------------|---|
| 0.01                     | 5.4   |
| 0.02                     | 10.8  |
| 0.1                      | 54.0  |
| 0.5                      | 270.0   |
| 1.0                      | 540.0   |

Add the required amount of 1+1 alkali to 10 liters of CO<sub>2</sub>-free H<sub>2</sub>O. Check the normality, which should be slightly strong, as directed below, and adjust to desired strength by the following formula:  $V_1 = V_2 \times N_2 / N_1$ , where  $N_2$  and  $V_2$  represent the normality and volume of stock soln, respectively, and  $V_1$  the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ . Determine the exact strength of the final soln as directed below.

STANDARDIZATION<sup>1</sup>

Accurately weigh sufficient dried acid potassium phthalate to titrate approximately 40 cc and transfer to a 300 cc flask which has been swept free from CO<sub>2</sub>. Add 50 cc of cool CO<sub>2</sub>-free H<sub>2</sub>O. Stopper the flask and swirl gently until the sample is dissolved. Add 3 drops of a 1% soln of phenolphthalein and titrate with the soln that is being standardized.

Calculate the normality (N) of the standard soln by the following formula:

$$N = \frac{\text{g potassium acid phthalate}}{\text{cc NaOH} \times 204.136/1000}$$

The normality value is exact only when phenolphthalein is used as an indicator.

## STANDARD SOLUTIONS OF HYDROCHLORIC ACID

## PREPARATION OF STANDARD SOLUTIONS

The following table gives the approximate amount of HCl (reagent quality, 35%-37% HCl) necessary to make 10 liters of standard solutions:

| <i>Approx. Normality</i> | <i>HCl to be diluted to 10 liters</i><br><i>cc</i> |
|--------------------------|--|
| 0.01                     | 8.9  |
| 0.02                     | 17.8   |
| 0.10                     | 89.0   |
| 0.50                     | 445.0  |
| 1.0                      | 890.0  |

## STANDARDIZATION

Titrate 40 cc against a standard alkali soln of approximately the same strength as the acid being standardized as directed under Sodium Hydroxide, using phenolphthalein as an indicator.

Determine the normality by the following formula:

$$N = \frac{\text{cc standard alkali} \times \text{normality of alkali}}{\text{cc HCl}}$$

<sup>1</sup> U. S. Bur. Standards Certificate of Analyses of Standard Sample No. 84.

If stronger than desired, dilute the soln to a definite normality value by the following formula:

$$V_1 = \frac{V_2 \times N_2}{N_1}, \text{ where}$$

$N_2$  and  $V_2$  represent the normality and volume of stock solution, respectively, and  $V_1$  represents the volume to which the stock soln should be diluted to obtain the desired normality,  $N_1$ .

Check the exact strength of the final soln by titration as directed above. The soln will be exact only if the same indicator is used in the determination and in the standardization.

If the standard acid solution is to be used with methyl orange as an indicator determine a correction for the volume of acid required to pass from the end point of phenolphthalein to that of methyl orange. Add<sup>1</sup> 3 drops of a 1% soln of phenolphthalein to 100 cc of  $\text{CO}_2$ -free  $\text{H}_2\text{O}$ , and then add sufficient alkali soln to give an end point with phenolphthalein. Disregard the quantity of alkali soln added and take the buret readings from this point. Add 3 drops of a 0.02% soln of methyl orange and sufficient 0.1  $N$  acid to produce the pink color of methyl orange. Titrate back with 0.1  $N$  alkali soln to the same end point taken in the usual titration (preferably  $\text{pH} = 4.2$ ). Buffered solns of 3.8, 4.0, and 4.2  $\text{pH}$  are useful in accurately determining the methyl orange end point. They may be prepared as follows:<sup>1</sup>

| <i>grams</i>  | <i>cc 0.1N</i> |
|---|----------------|
| $\text{pH} = 3.8$ 2.041 KH phthalate + 5.26 HCl. Dilute to 200 cc.  |                |
| $\text{pH} = 4.0$ 2.041 KH phthalate + 0.80 NaOH. Dilute to 200 cc. |                |
| $\text{pH} = 4.2$ 2.041 KH phthalate + 7.40 NaOH. Dilute to 200 cc. |                |

If the acid and alkali solns are equivalent, the quantity of acid minus the quantity of alkali soln represents the quantity of acid required to pass from the phenolphthalein end point to that of methyl orange.

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No report was given by the Committee on Standard Scale for Immersion Refractometer.

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## REPORT OF COMMITTEE TO CONFER WITH THE AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS OF MILK ANALYSIS

The specific duties of this Committee are to confer and cooperate with authorities acting for the American Public Health Association in the preparation of their text, Standard Methods of Milk Analysis. This involves primarily a transcript of our A.O.A.C. methods official at the time for the analysis of milk and cream to the end that the procedure of the two Associations shall be identical so far as these methods are concerned.

Your Committee collaborated on the above plan in the preparation of the latest edition (6th, 1934), of the A.P.H.A. text. No active collaborative project will confront this Committee until the time of the next revision of the A.P.H.A. text when again our A.O.A.C. methods will be supplied.

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<sup>1</sup> Clark, Determination of Hydrogen Ions, 2nd ed., p. 106 (1922):

As a matter of general policy it is most desirable that chemical and biochemical methods in the official texts of the two associations should be identical where the same tests and determinations are concerned, otherwise confusion is likely to arise in control work due to differences in methods and procedure. This was the thought that prompted efforts to insure uniformity in methods for testing milk and cream, and the same thought is valid wherever methods of mutual interest to the two associations are involved. The plan already inaugurated whereby one individual serves as referee for both associations is an ideal way to insure the desired uniformity; and such a plan is facilitated by a considerable overlapping in membership between the two groups. Your Committee has no desire to extend the scope of its activities beyond that specifically imposed upon it, but deems it proper to encourage the single-referee plan wherever feasible, as a means of avoiding confusion in food control work that will arise from divergent methods for the same examination or analysis, each being of official character.

E. M. BAILEY  
G. G. FRARY  
F. C. BLANCK

Approved.

#### REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PRO- TECTION INSTITUTE

In the report submitted in 1934 there was given a complete list, with objectives, of the research projects which were in progress at that time under the auspices of the Crop Protection Institute. All of these projects are being continued and the following new ones have been added during this year.

1. A fundamental detailed study of cuprous oxide as a means of control of various plant diseases. This project is being conducted in cooperation with the Geneva, New York, Experiment Station.

2. A "DX Project," which involves a detailed study of new contact sprays. The headquarters of this project is located at the New Hampshire Experiment Station. The work in New Hampshire is supplemented by field work located in Florida and Delaware.

3. A preliminary study of various new copper compounds which might be used as fungicides is being made in cooperation with the Illinois Experiment Station.

4. The full time of one man is now devoted to a study of some new organic compounds which give promise as insecticides and fungicides. This project is being conducted at the Illinois Experiment Station.

5. In cooperation with the Indiana Experiment Station and the General Chemical Company the Institute has conducted extensive and in-

teresting studies at a number of places on the use of substitutes for lead arsenate for insect control.

The Institute can record the successful development of dilute sulfuric acid in weed control. Large scale field tests, last spring, in California gave significant results at economical cost. Suitable power apparatus for the application of the dilute acid has been developed. A comprehensive report on this project is in print.

The Institute also can record the successful development of new organic compounds for spraying for the control of codling moth.

Persons desiring further details concerning any of these projects should communicate with the chairman of the Institute, Dr. W. C. O'Kane, Durham, New Hampshire.

H. J. PATTERSON  
W. H. MACINTIRE

Approved.

## REPORT OF SECRETARY-TREASURER

The first matter of importance in the report of the Secretary is the adoption or action on the proposed amendments to the Constitution and By-laws. As provided in the Constitution, these amendments were read Monday morning and submitted to the Executive Committee. The amendment to the Constitution is to that part of Article 3 which reads as follows:

With the concurrence of the Executive Committee, the president shall also appoint a chairman and a committee of nine other members, which shall be designated as a Committee on Recommendations of Referees, one-third of the membership of which shall be appointed at intervals of two years to serve six years, the chairman to be appointed annually. The chairman shall divide the nine members into subcommittees (A, B, and C) and shall assign to each subcommittee the reports and subjects which it shall consider.

The recommendation is that the *nine* members designated shall be changed to *twelve*. The adoption of this amendment was unanimous.

By-law 10 reads as follows:

Each department, college, experiment station, board, or other institution entitled to representation in the Association shall contribute annually \$5.00 prior to the first of January following the regular annual meeting.

The financial condition of the Association is such that in the opinion of the Executive Committee the assessment of \$5.00 can be waived, and it has been recommended that By-law 10 be deleted.

It was regularly moved, seconded, and carried that these changes be made in the Constitution and By-laws.

During the year it was found necessary to appoint a number of additional referees. These names were published in later editions of *The Journal*. You have heard reports from most of these referees this session,

It was decided by the Executive Committee to recommend to this meeting the appointment of a Referee on Disinfectants. I move that an associate referee on disinfectants be approved by the Association. (Motion was seconded and carried.)

I have already reported on the supply of the *Book of Methods* still on hand, approximately 750 copies. The demand for this book recently has been about 50 copies per month. As I have also reported, it has been decided by the Executive Committee to offer this book for sale at \$2.50 per copy. The Committee on Revision hopes to have the new *Book of Methods* ready for distribution before July 1, 1936.

After some discussion, the Executive Committee decided to discontinue the Committees on Sampling and on Bibliography.

The Executive Committee passed the usual expense account for the meeting and approved the auditing fee. The accounts of the Association are now audited by a certified accountant. The Executive Committee also approved the necessary traveling expenses of Miss Lapp in connection with the revision of the *Book of Methods*. I shall now read the summarized statement of the expense account from the balance sheet. The statement pictures a gratifying financial status, and makes it possible for us to pay cash on delivery for our next *Books of Methods*. On previous orders it has been necessary for our publisher to be our banker in that he carried the copies, permitting us to draw such numbers as were needed for sale.

#### CASH RECEIPTS AND DISBURSEMENTS

YEAR ENDED SEPTEMBER 30, 1935

*Balance, October 1, 1934:*

|   |            |            |
|---|------------|------------|
| Lincoln National Bank.....                    | \$4,851.77 |            |
| Montgomery Building and Loan Association..... | 2,276.46   | \$7,128.23 |

#### RECEIPTS

*Sales:*

|                         |           |            |
|-------------------------|-----------|------------|
| Reprints.....           | \$ 139.79 |            |
| Journals.....           | 3,935.70  |            |
| Methods.....            | 3,620.15  |            |
| Wiley's Principles..... | 35.00     | \$7,730.64 |

Less: Discounts Allowed:

|               |           |        |
|---------------|-----------|--------|
| Journals..... | \$ 441.62 |        |
| Methods.....  | 416.75    | 858.37 |

Net Sales..... 6,872.27

*Other Income:*

|   |        |        |
|---|--------|--------|
| Premiums on foreign exchange.....               | \$ .82 |        |
| Membership dues.....                            | 250.00 |        |
| Advertising.....                                | 225.00 |        |
| Interest—U. S. Treasury Bonds.....              | 175.65 |        |
| Interest—Montgomery Building and Loan Ass'n.... | 276.19 |        |
| Interest—Federal Land Bank Bonds.....           | 21.25  |        |
| Liquidating dividend—Brentano's, New York.....  | .27    | 949.18 |

*Miscellaneous:*

|   |         |                    |
|---|---------|--------------------|
| Returned checks made good .....         | \$ 9.00 |                    |
| Books ordered through Association ..... | 296.68  | 305.68             |
|   |         | <u>\$15,255.36</u> |

## DISBURSEMENTS

*Expenses:*

|                                       |           |
|---------------------------------------|-----------|
| Salaries .....                        | \$ 995.00 |
| Postage and box rent .....            | 370.00    |
| Meeting and Association expense ..... | 467.32    |
| Traveling expense .....               | 82.86     |
| Stationery and supplies .....         | 171.84    |
| Joseph Cohen, affidavits .....        | 10.00     |
| Auditing .....                        | 130.00    |
| Check tax and exchange .....          | 2.79      |

## Printing:

|                                  |           |             |
|----------------------------------|-----------|-------------|
| Reprints .....                   | \$ 281.00 |             |
| Journals .....                   | 3,041.02  |             |
| Anniversary booklet .....        | 652.62    |             |
| Miscellaneous .....              | 24.15     | \$3,998.79  |
| Binding and storage .....        | 15.00     |             |
| Commissions and brokerage .....  | 12.50     |             |
| Samples .....                    | 1.03      |             |
| Premiums, employees' bonds ..... | 10.00     |             |
| Safe deposit rental .....        | 3.30      |             |
| Sales refunds .....              | 1.00      | \$ 6,271.43 |

*Capital Expenditures:*

|   |            |          |
|---|------------|----------|
| Purchase of Home Owners Loan Corporation Bonds .. | \$1,000.00 |          |
| Purchase of Federal Land Bank Bonds .....         | 4,000.00   |          |
| Bond interest and discount .....                  | 95.71      | 5,095.71 |

*Miscellaneous:*

|   |           |        |
|---|-----------|--------|
| Books ordered through Association ..... | \$ 296.68 |        |
| Returned checks .....                   | 15.00     | 311.68 |

## BALANCE, SEPTEMBER 30, 1935

|  |            |                    |
|--|------------|--------------------|
| Lincoln National Bank .....                    | \$2,023.89 |                    |
| Montgomery Building and Loan Association ..... | 1,552.65   | 3,576.54           |
|  |            | <u>\$15,255.36</u> |

W. W. SKINNER

Approved.

No report was given by the Committee to Cooperate with Other Committees on Food Definitions because no meeting of the Food Standards Committee was held during the year.

No report was given by the Committee on Sampling.

No report was given by the Committee on Bibliography.



## REPORT OF AUDITING COMMITTEE

The Auditing Committee examined the public accountant's report of the books of the Association of Official Agricultural Chemists, Inc., as of September 30, 1935, and found it to be correct. The bonds on deposit and the active bank balances were also verified.

L. S. WALKER

J. J. T. GRAHAM

Approved.

## REPORT OF COMMITTEE ON NECROLOGY

The greatest loss among the membership of the Association to be recorded since the previous meeting is the death of Dr. James Monroe Bartlett, who passed away after a very brief illness in the Eastern Maine General Hospital of Bangor, Maine, on May 11, 1935, at the advanced age of 79 years. Dr. Bartlett was born in Litchfield, Maine, and graduated from the Maine State College in 1880 with the degree of B.S. in chemistry. After a short post graduate course at Cornell, he obtained his M.S. degree from Maine in 1883. The following two years he was Assistant Chemist at the Pennsylvania State College Agricultural Experiment Station. On May 1, 1885, he began his long service of exactly fifty years and ten days in the Chemical Department of the Maine Agricultural Experiment Station, first as Assistant and shortly thereafter as Chief Chemist. During this period he served twice as Acting Director of the Maine Station, but declined to accept this office as a permanent appointment. While the greater part of his experiment station activities was confined to the miscellaneous regulatory control of fertilizers, cattle feeds, insecticides, foods, and drugs, he nevertheless gave considerable attention to various research projects upon cattle and poultry feeding, and upon variety tests and fertilizing and spraying experiments with potatoes.

Dr. Bartlett had taken an active interest in the work of our Association since the time of his first attendance at the 8th Annual Meeting in 1891. Although not a constant attendant he participated in the work of twenty of our meetings. At the 1894 and 1895 meetings he was reporter on nitrogen and at the 1908 and 1909 meetings referee on dairy products. He was a member of the Executive Committee in 1910. At the 1911 meeting he served as chairman of the Auditing Committee and at the 1913 meeting as Chairman of the Committee on Resolutions. In 1917 he was the Associate Referee on Tea and Coffee. Dr. Bartlett's most conspicuous work for the Association was his working out of an emergency method for determining borax in fertilizers at a time when some of our domestic supplies of potash were contaminated with this impurity. This work is comprised in his paper on "The Distillation Method for the

Estimation of Borax in Mixed Fertilizers" in the Proceedings for 1920 and in his reports on "Borax in Fertilizers" for the 1922 and 1923 meetings. His obituary of Dr. Charles D. Woods was published in the Journal of the Association for August 1926.

In recognition of his long service in behalf of the agricultural welfare of his native state, Dr. Bartlett was given the honorary degree of Doctor of Science by the University of Maine in 1927.

Dr. Bartlett exercised a great influence at the meetings of our Association not only by his papers and reports but by his constant helpful advice upon a large number of the referee projects in which he served as collaborator. He was a conspicuous figure at the semi-centennial anniversary of our Association in 1934, and the many members who met him then for the last time will long retain the memory of his kindly genial personality.

A full account of Dr. Bartlett's interesting career by President-Elect H. H. Hanson will be published in one of the forthcoming issues of our *Journal*.

Another familiar figure at our annual gatherings and a former member of the Association, who passed away since our last meeting, was John Sharkey Carroll, who died on September 15, 1935. He was born in Oktibeha County, Mississippi, in 1871, and studied agriculture at the Mississippi Agricultural College, where he took the degree of B.S. in 1892 and of M.S. in 1896, in which year he was appointed Assistant Professor of Chemistry in the Mississippi Agricultural College and Assistant State Chemist with work relating to the inspection and analyses of fertilizers. In 1904 he accepted the position of local manager of educational and scientific work of the German Potash Syndicate at Atlanta, Georgia, which he filled until the period of the late war. Of late years Mr. Carroll had been connected with the agricultural and scientific work of various potash interests and at the time of his death was manager of the Southwest Territory for the American Potash Institute. His many friends in the Association will regret to learn of his passing.

C. A. BROWNE  
H. H. HANSON  
H. C. LYTHGOE

Approved.

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## REPORT OF NOMINATING COMMITTEE

The Committee on Nominations submits the following report:

*President:* H. H. Hanson, Dover, Del.

*Vice-President:* C. C. McDonnell, Washington, D.C.

*Secretary-Treasurer:* W. W. Skinner, Bureau of Chemistry and Soils, Washington, D.C.

*Additional Members of the Executive Committee:*

H. R. Kraybill, Lafayette, Ind.  
W. S. Frisbie, Washington, D.C.  
C. L. Hare, Auburn, Ala.

*Post-Officio:*

F. C. Blanck, Washington, D.C.

H. A. LEPPER  
O. B. WINTER  
W. H. MACINTIRE

A unanimous vote was cast for the officers nominated.

H. A. Lepper was reappointed Chairman of the Committee on Recommendations of Referees; G. E. Grattan was appointed on Sub-committee A to fill the unexpired term of L. E. Bopst, resigned; J. W. Sale, Chairman; J. A. LeClerc and W. C. Jones were appointed as members of the newly formed Subcommittee D. L. S. Walker was appointed Chairman of the Committee on Definition of Terms and Interpretation of Results on Fertilizers and Liming Materials, and W. C. Jones was appointed in the place of J. W. Kellogg on this committee.

Mr. Lythgoe escorted Mr. Hanson to the Chair.

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*Mr. Hanson:* Mr. President, members of the Association: I cannot claim that this is entirely a surprise because rumors of the impending action reached my ears. However, when I think of the long line of eminent agricultural chemists who have filled this office, I assure you that I accept it with great humility, promising only that I will do my best for you, hoping that you will overlook shortcomings, and well knowing that our excellent secretary and his very efficient assistants will do their best to keep me in the straight and narrow path. I am deeply grateful I assure you.

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## REPORT OF COMMITTEE ON RESOLUTIONS

*Whereas*, this, the fifty-first annual meeting of our Association of Official Agricultural Chemists, has exceeded in attendance record all previous meetings; and *whereas*, the excellent program has been carried out with despatch and the sustained interest and participation of our membership, be it therefore.

*Resolved:* That the Association extend to its president, Dr. F. C. Blanck, its Vice-President, Mr. H. H. Hanson, its Secretary-Treasurer, Dr. W. W. Skinner, and Assistant Secretary, Miss Marian E. Lapp, and to the other members of its executive committee this expression of appreciation for their most excellent preparation for, and conduct of, the meeting.

*Resolved:* That the Association extend to its retiring president, Dr. Blanck, its sincere appreciation of his scholarly presidential address and of his fairness and efficiency as its presiding officer.

*Resolved:* That the Association express its sincere thanks to Dr. W. H. MacIntire for his timely and valuable Wiley Memorial Address on "Certain Practical Aspects of Soil Chemistry Research."

*Resolved:* That the Association convey through its Secretary to the management of the Raleigh Hotel this expression of its appreciation of the very real assistance rendered toward the success of the meeting by furnishing all necessary accommodations for our gathering, and for the many courtesies extended our members and guests.

GUY G. FRARY  
H. R. KRAYBILL  
J. W. SALE

Approved.

## CONTRIBUTED PAPERS

### AN IMPROVEMENT ON THE GROSS AND SMITH COLORIMETRIC METHOD FOR THE DETERMINATION OF ROTENONE AND DEGUELIN

By LYLE D. GOODHUE, Division of Insecticide Investigations,  
Bureau of Entomology and Plant Quarantine, U. S.  
Department of Agriculture, Beltsville, Md.)

Gross and Smith<sup>1</sup> have reported a colorimetric method for the quantitative determination of rotenone and deguelin which depends on the development of a red color proportional to the sum of the amounts of these two compounds. This method consists of treating an acetone solution of the sample with alcoholic potassium hydroxide and after a suitable interval acidifying with dilute nitric acid containing a trace of sodium nitrite. The procedure is not entirely satisfactory, chiefly because an intense yellow background is introduced by the reagents. Other difficulties such as instability and variation of color were also encountered by the writer during the analysis of derris extracts.

In this paper changes are proposed which practically eliminate these difficulties. Sulfuric acid is substituted for nitric acid, the alcoholic potassium hydroxide is diluted with water, and the nitrite, which is necessary to produce the red color, is added with the alcoholic potassium hydroxide.

#### REAGENTS

(a) *Sulfuric acid solution*.—Mix 1 volume of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84) with 3 volumes of water. The  $\text{H}_2\text{SO}_4$  should be free from nitrous acid.

(b) *Alcoholic solution of sodium nitrite*.—Dissolve 1 gram of sodium nitrite in 10 cc. of water and dilute to a volume of 1 liter with 95 per cent alcohol.

(c) *Potassium hydroxide solution*.—Dissolve 40 grams in 100 cc. of water.

(d) *Alkali solution*.—Mix 1 volume of reagent (c) and 7 volumes of reagent (b). Prepare this solution fresh daily. This solution could be prepared by adding the sodium nitrite to the stock solution of potassium hydroxide and then diluting with 95 per cent alcohol, but there is some doubt about the stability of sodium nitrite in strong alkalis over a period of time.

#### PROCEDURE

Prepare an acetone extract of the sample containing from 0.005 to 0.25 mg. of rotenone per cc. and pipet 2 cc. into a dry test tube. Add 2 cc. of the alcoholic potassium hydroxide solution, and place the tube in a water bath at about 25° C. for 5 minutes. Add 5 cc. of the dilute sulfuric acid solution, stopper, shake, and place the tube back in the water bath. The color reaches a maximum after about 15 minutes, and then remains unchanged for 2 hours. Determine the amount of rotenone by comparing the color with standards prepared at the same time from known quantities of rotenone.

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<sup>1</sup> *This Journal*, 17, 336-339 (1934).

The turbidity or brown color which sometimes develops during the analysis of crude plant extracts may be removed by extracting the final mixture with a small portion of ether. The red color due to rotenone and deguelin is not extracted.

### DISCUSSION

In the original test the amount of nitrite is difficult to control and hence the color, since the hue and depth of color depend on the nitrite concentration. By diluting the alcoholic potassium hydroxide with water and by introducing the nitrite through this reagent instead of the acid, the color produced by the rotenone as well as that introduced by the reagents themselves can easily be controlled.

At 25° C. the minimum time to allow for the alcoholic potassium hydroxide to react is 4 minutes. Very little if any increase in color is noted after 15 minutes. The temperature may be between 20° and 30° C. if both the standards and the unknowns are prepared at the same temperature. If the procedure is carried out below 20° C. the amount of sodium nitrite must be increased.

Table 1 gives the amount of red color produced by different amounts of rotenone. The comparisons were made with Lovibond color slides in a LaMott roulette comparator. The red color was more easily matched when the small amount of blue which accompanies the red was filtered out by a dilute dichromate solution. The test was carried out at 27° C. and the tubes were held at this temperature during the period of observation. No red and only a trace of yellow appeared in the blank.

TABLE 1.—Amount of red color as measured on the Lovibond scale from different quantities of rotenone

| ROTENONE | UNITS OF RED COLOR ON THE LOVIBOND SCALE AT 27° C. |             |              |                |              |
|----------|--|-------------|--------------|----------------|--------------|
|          | INITIAL <sup>1</sup>                               | AFTER 1 HR. | AFTER 2 HRS. | AFTER 3.5 HRS. | AFTER 5 HRS. |
| mg.      |  |             |              |                |              |
| 0.01     | 0.5  | 0.5         | 0.5          | 0.4            | 0.3          |
| 0.05     | 2.5  | 2.5         | 2.5          | 2.0            | 1.5          |
| 0.10     | 4.5  | 4.5         | 4.5          | 4.0            | 3.5          |
| 0.30     | 12.0   | 12.0        | 12.0         | 11.0           | 10.0         |
| 0.50     | 18.0   | 18.0        | 18.0         | 16.0           | 15.0         |

<sup>1</sup> The initial reading was taken after allowing 20 minutes for the colors to develop.

The specificity of the test remains the same as the original. Deguelin gives the same amount of color as rotenone, while tephrosin and toxicarol do not give the test. Derris samples gave about twice the amount of rotenone as indicated by the Jones<sup>1</sup> method, probably due to the deguelin present.

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 23 (1933).

## SUMMARY

The Gross and Smith colorimetric method for the determination of rotenone and deguelin has been improved. Sulfuric acid has been substituted for nitric acid, the concentration of the alcoholic potassium hydroxide has been reduced by diluting with water, and the nitrite necessary to produce the color is added as sodium nitrite in the alcoholic potassium hydroxide. The stability, hue, and depth of color produced by the rotenone as well as that produced by the reagents themselves can be easily controlled. This combination of reagents practically eliminates fading and increases the sensitivity of the test 20 times.

## DETERMINATION OF THUJONE IN ABSINTH-TYPE LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.)

No reagent has been found that will yield a compound sufficiently insoluble in alcohol or with sufficient stability to withstand the treatment necessary to separate thujone from the large proportions of alcohol contained in absinth-type liqueurs.

Unfortunately, ketones do not react in the uniform manner expected of aldehydes, so that any predictions as to their behavior often go awry. It is also difficult to obtain pure substances, and therefore supposedly standard solutions are often not really standard.

Semicarbazide is the reagent used most frequently for the purpose of holding back thujone. However, the experiments of the writer indicate that thujone semicarbazone is not sufficiently stable for the purposes of a quantitative method.

The qualitative method in use today, that of Enz,<sup>1</sup> involves a modification of the Legal test.<sup>2</sup> The details follow:

To 500 cc. of absinth add 1 cc. of freshly distilled aniline and 1 cc. of sirupy phosphoric acid, and reflux 30 minutes on the steam bath. Distil off two 100 cc. portions, reject the first, and test the second for thujone as follows:

Add 0.5 gram of semicarbazide hydrochloride and 0.6 gram of anhydrous sodium acetate (or 1.0 gram of the crystallized salt) and allow the mixture to stand overnight. Distil off the alcohol at as low a pressure as possible. Steam distil to remove essential oils and other volatile material, collect, and reject about 15 cc. of distillate. Wash down the condenser with a little alcohol and with water. Cool the sample, add 1 cc. of  $\text{H}_2\text{SO}_4(1+1)$ , and again steam distil, this time collecting 20 cc. of distillate in a cylinder. Pour the distillate into a small separatory funnel, and add 20 cc. of ether, using the receiver as the measure. Shake and separate the ether solution. Add 10 cc. of 65 per cent alcohol and allow the ether to evaporate spontaneously. When all the ether has evaporated, note the odor of the residue. The odor of thujone will be apparent if 2 mg. or more is present in the solution, provided it is

<sup>1</sup> *Schweiz. Wochschr.*, 49, 337, 507 (1911).

<sup>2</sup> *Rocques, Ann. chim. anal.*, 13, 227 (1908).

not masked by the presence of other odoriferous substances. Make the modified Legal test as follows:

To the solution obtained as directed above, add 1 cc. of 10 per cent zinc sulfate solution and 0.25 cc. of freshly prepared sodium nitroprusside solution (0.1 gram per cc. of  $H_2O$ ). Slowly, with constant stirring, add 2 cc. of 5 per cent sodium hydroxide solution. Allow to stand one or two minutes. Add 1.5 cc. of glacial acetic acid and mix. A precipitate of raspberry red color (resembling the alcohol precipitate of a red fruit juice) shows the presence of thujone. A negative test is shown by a similar precipitate having an appearance similar to that of the alcohol precipitate from apple jelly or other light colored fruit.

The above procedure was studied critically. First the modified Legal test was applied to solutions of known quantities of thujone in 10 cc. of 65 per cent alcohol. The results are given in Table 1.

TABLE 1.—*Results, modified Legal test*

| THUJONE PRESENT | TEST              | THUJONE ODOR |
|-----------------|-------------------|--------------|
| mg.             |                   |              |
| 1               | Doubtful          | None         |
| 1               | Positive          | None         |
| 2               | Positive          | Slight       |
| 2               | Doubtful          | Slight       |
| 5               | Strongly positive | Strong       |
| 5               | Strongly positive | Strong       |
| 10              | Strongly positive | Strong       |
| 10              | Strongly positive | Strong       |

Known quantities of thujone were then placed in the steam distillation apparatus, and 25 cc. of water was added. The solution was steam distilled, and 20 cc. of distillate was collected. The distillate was extracted with ether, and the test was made on the ether solution. The results are given in Table 2.

TABLE 2.—*Recovery of thujone by steam distillation*

| THUJONE PRESENT | THUJONE ODOR | TEST              |
|-----------------|--------------|-------------------|
| mg.             |              |                   |
| 1               | None         | Doubtful          |
| 1               | None         | Doubtful          |
| 2               | Slight       | Positive          |
| 2               | Slight       | Positive          |
| 5               | Strong       | Strongly positive |
| 5               | Strong       | Strongly positive |

These experiments show that positive results may be expected from the modified Legal test if 2 mg. or more of thujone is present in the solution when steam distilled, and also that the odor of thujone can be detected when 2 mg. or more is present in the 10 cc. of solution to be tested.



For convenience the semicarbazide reagent was made up by dissolving 10 grams of semicarbazide hydrochloride and 12 grams of anhydrous sodium acetate in water and diluting to 100 cc. Known quantities of thujone were added to 100 cc. portions of 80 per cent alcohol, 5 cc. of semicarbazide reagent was added to each, and the mixtures were allowed to stand overnight. The next day the alcohol was distilled off by means of a Widmer distilling apparatus at a pressure of about 60 mm., the distilling flask being heated with a water bath. The temperature of the vapors was about 35° C. When only about 15 cc. of liquid remained, the flask was disconnected from the Widmer column,<sup>1</sup> about 10 cc. of water was added, and the flask was connected to a steam distillation apparatus and steam distilled. About 15 cc. of distillate was collected in a 25 cc. graduated cylinder. The receiver was then removed, and while the distilling flask was allowed to cool, the condenser was washed down with two small portions (about 10 cc. each) of alcohol and two portions of water. The washings were rejected. About 1 cc. of H<sub>2</sub>SO<sub>4</sub> (1+1) was then added to the distilling flask, and the contents were mixed. The liquid was again steam distilled, and 20 cc. of distillate was collected in a 25 cc. graduated cylinder. The distillates were extracted with 20 cc. of ether, 10 cc. of 65 per cent alcohol was added to the ether extract, and the mixture was allowed to evaporate spontaneously until the odor of ether could no longer be detected. The modified Legal test was then applied to the residue after its odor had been noted. The results are given in Table 3.

TABLE 3.—*Thujone held back as semicarbazone during removal of alcohol*

| THUJONE PRESENT | 1ST DISTILLATION BEFORE ACIDIFYING |                      | 2ND DISTILLATION AFTER ACIDIFYING |          |
|-----------------|------------------------------------|----------------------|-----------------------------------|----------|
|                 | ODOR                               | TEST                 | ODOR                              | TEST     |
| mg.             |                                    |                      |                                   |          |
| 1               | None                               | Negative             | None                              | Doubtful |
| 2               | None                               | Negative             | Doubtful                          | Positive |
| 3               | None                               | Negative             | Thujone                           | Positive |
| 5               | None                               | Negative             | Thujone                           | Positive |
| 10              | Thujone                            | Slightly<br>positive | Strong<br>thujone                 | Positive |
| 20              | Doubtful                           | Negative             | Strong<br>thujone                 | Positive |
| 50              | Thujone                            | Doubtful             | Strong<br>thujone                 | Positive |

The data in Table 3 show that thujone semicarbazone has sufficient stability to withstand the removal of alcohol and essential oils and to give a positive test for thujone in the proper fraction when 2 mg. is present, although 3 mg. or more must be present before the odor of thujone is apparent in the fraction. The data also show that when 10 mg. or more

<sup>1</sup> *Helv. Chim. Acta*, 7, 59 (1924).

of thujone is present, a sufficient quantity may be lost in the steam distillation before the thujone semicarbazone is decomposed with acid, so that thujone may be detected both by odor and by the modified Legal test in that distillate. This behavior precludes the possibility of using semicarbazide as a means of holding back thujone quantitatively.

Next, known quantities of thujone were added to 500 cc. portions of 50 per cent alcohol and distilled slowly. Two 100 cc. fractions were collected, each of which was treated in the same manner as were the solutions in the previous experiment. The results are given in Table 4.

TABLE 4.—*Detection of thujone in solutions by the modified Enz method*

| THUJONE PRESENT | FRACTION | 1ST DISTILLATION BEFORE ACIDIFYING |          | 2ND DISTILLATION AFTER ACIDIFYING |          |
|-----------------|----------|------------------------------------|----------|-----------------------------------|----------|
|                 |          | THUJONE ODOR                       | TEST     | THUJONE ODOR                      | TEST     |
| mg.             |          |                                    |          |                                   |          |
| 1               | a        | None                               | Negative | None                              | Negative |
|                 | b        | None                               | Negative | None                              | Negative |
| 2               | a        | None                               | Negative | None                              | Negative |
|                 | b        | None                               | Negative | Doubtful                          | Negative |
| 3               | a        | None                               | Negative | Slight                            | Doubtful |
|                 | b        | None                               | Doubtful | None                              | Positive |
| 5               | a        | None                               | Negative | Slight                            | Negative |
|                 | b        | None                               | Positive | Slight                            | Positive |
| 10              | a        | Doubtful                           | Negative | Slight                            | Positive |
|                 | b        | Slight                             | Positive | Slight                            | Positive |
| 20              | a        | None                               | Negative | Slight                            | Negative |
|                 | b        | None                               | Negative | Distinct                          | Sl. pos. |
| 50              | a        | None                               | Negative | Distinct                          | Positive |
|                 | b        | Doubtful                           | Positive | Strong                            | Positive |
| 100             | a        | Doubtful                           | Positive | Strong                            | Positive |
|                 | b        | Distinct                           | Positive | Strong                            | Positive |

The data in Table 4 show that when the modified Enz method is used a positive reaction for thujone may be expected in the proper fraction when 3 mg. or more is present in the sample. In most cases the odor of the fraction appears to be as good an index of the presence of thujone as is the test, but the test and odor may serve as confirmatory evidence of the presence of thujone, especially when less than 10 mg. is present.

The experience of the writer indicates that less than 3 mg. of thujone gives a positive test at times but not always. Before the Widmer column was obtained a variety of kinds of apparatus was used for removing alcohol, but the results were irregular. In some cases a positive test would be obtained when 2 mg. was present, and sometimes negative tests were obtained when 5 mg. or more thujone was present.

In the testing of commercial samples of absinth-type liqueurs, the color test is sometimes more or less obscured by an olive green color of unknown origin.

The minimum and maximum quantities of thujone to be expected in absinth were calculated from the literature. A study of books on the manufacture of cordials shows that when they are made from essences from 0.6 to 1.0 gram of wormwood oil is added for each 10 liters of absinth. As wormwood oil is reported to contain from 3 to 45 per cent of thujone, the thujone in absinth may vary from 1.8 mg. to 45 mg. per liter.

The available recipes indicate that when absinth is made from the plant, from 25 to 32 grams of the herb *artemisia absinthium* are extracted for each liter of the liqueur. Since about 0.3 per cent of essential oil containing from 3 to 45 per cent of thujone is obtained from the herb, it may be expected that absinth made from the herb will contain from 2 to 34 mg. of thujone in one liter. The study of the modified Enz method recorded here would indicate that while positive tests may be expected on absinth-type liqueurs containing the larger quantities of oil or extracted herb, many of these products made with smaller quantities of oil or herb extract would escape detection.

#### SUMMARY

While the present available procedures for the detection of thujone in absinth-type liqueurs can be depended upon to give positive tests when 5 mg. or more of the ketone is present, the fact that the test is negative does not show conclusively that the liqueur does not contain thujone in quantities which may be regarded as significant from a pharmacological point of view.

#### ADDITIONAL REFERENCES

- (1) Anonymous, Determinations Required under the Swiss Absinth Law. *Veröffentl. kais. Gesundh.*, 34, 1205 (1910).
  - (2) Charabot and Lalone, Successive Distribution of Terpene Compounds in the Various Organs of the Living Plant. *Compt. rend.*, 144, 152, 435 (1907).
  - (3) Cuniassé, Characterization of the Essence of Absinth. *Bull. soc. chim.* (4) 1, 279 (1907).
  - (4) Delage, The Masking of the Ketone Reaction for Essential Oils in Absinth and Similar Liqueurs. *Ann. fals.*, 16, 412 (1923).
  - (5) Duparc and Monnier, Identification of Thujone in Liqueurs. *Ann. chim. anal.*, 13, 378 (1908).
  - (6) Phillipe and von Fallenburg, The Work of H. Enz on the Testing of Thujone in Absinth. *Schweiz. Wochschr.*, 49, 418 (1911).
  - (7) Ronnet, Analysis of Commercial Absinth. *Ann. fals.*, 3, 477 (1910).
  - (8) Schaffer and Phillipe, Investigation of and Standards for Absinth and its Substitutes. *Mitt. Lebensm. Hyg.*, 1, 1 (1910).
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## NOTES ON THE PERMANGANATE METHOD OF ESTIMATING REDUCED COPPER IN THE DETERMINATION OF REDUCING SUGARS\*

By H. R. KRAYBILL, W. J. YOUDEN, and J. T. SULLIVAN

The permanganate method for estimating reducing sugars is used extensively in biological work in this country and in European countries. Frequently a combination of the permanganate method for estimating the reduced copper and some other method such as the Munson and Walker<sup>1</sup> or Quisumbing and Thomas method<sup>2</sup> for reducing the copper is used.

Various workers<sup>3</sup> state that it is necessary to standardize the permanganate against the cuprous oxide produced by a known quantity of pure glucose. It has been claimed that when oxalic acid, sodium oxalate, or iron is used too low results are obtained.

The writers have standardized solutions of permanganate with Bureau of Standards sodium oxalate in acid solution and with weighed samples of cuprous oxide prepared by reducing with invert sugar (prepared by acid hydrolysis of sucrose) according to the conditions of the Munson and Walker method and obtained excellent agreement by the two methods. Excellent agreement was also obtained between the amount of copper determined by the permanganate method and the amount found electrolytically in the final solutions after the copper had been determined by the permanganate method.

### STANDARDIZATION OF POTASSIUM PERMANGANATE WITH SODIUM OXALATE AND WITH CUPROUS OXIDE

The potassium permanganate solutions were prepared by dissolving the salt in water, allowing the solution to stand in the dark for several weeks, and then filtering through asbestos. The cuprous oxide was prepared by heating invert sugar, obtained from the acid hydrolysis of sucrose, with Fehling's solution according to the conditions of Munson and Walker. Analysis of the cuprous oxide by the electrolytic method showed that it contained the theoretical amount of copper. The ferric ammonium sulfate solution contained 240.9 grams of the crystalline salt and 200 cc. of concentrated sulfuric acid per liter.

The permanganate solutions were standardized by use of Bureau of Standards sodium oxalate according to the method of McBride.<sup>4</sup> They were also standardized by use of weighed samples of the cuprous oxide as follows: The sample of cuprous oxide was placed in a 400 cc. beaker;

\* Contribution from The Boyce Thompson Institute, Inc., Yonkers, N. Y. Presented at the Annual Meeting of the Association of Official Agricultural Chemists, November, 1930.

<sup>1</sup> *J. Am. Chem. Soc.*, 28, 663 (1906).

<sup>2</sup> *Ibid.*, 43, 1503 (1921).

<sup>3</sup> *Methods of Analysis*, A.O.A.C. 1895, U. S. Dept. Agr. Div. Chem. Bull. 46; Bur. Standards Cir. 44, p. 88 (1918); *Methods of Analysis*, A.O.A.C., 1925, 192; *J. Am. Chem. Soc.*, 46, 1662-69 (1924); *This Journal*, 17, 293 (1934).

<sup>4</sup> *J. Am. Chem. Soc.*, 34, 415 (1912).

TABLE 1.—Standardization of 0.05 N potassium permanganate

| STANDARD   | WEIGHT  | TITRATION | CORRECTION<br>FACTOR | TEMPERATURE | CORRECTED<br>TO 25° | DIFFERENCE |
|------------|---------|-----------|----------------------|-------------|---------------------|------------|
|            | grams   | cc.       |                      | °C.         |                     | per cent   |
| Solution 1 |         |           |                      |             |                     |            |
| Sodium     | 0.12237 | 33.66     | 0.9965               | 23          | 0.9936              |            |
| Oxalate    | 0.12722 | 38.09     | 0.9970               | 23          | 0.9944              |            |
|            | 0.13497 | 40.44     | 0.9963               | 23          | 0.9938              |            |
|            | 0.13030 | 38.96     | 0.9983               | 18.5        | 0.9974              |            |
|            | 0.12295 | 36.79     | 0.9976               | 18.5        | 0.9967              |            |
|            | 0.13317 | 39.78     | 0.9993               | 18.5        | 0.9984              |            |
|            |         |           |                      |             | Av. 0.9957          |            |
| Cuprous    | 0.12492 | 35.07     | 0.9954               | 23          | 0.9954              |            |
| Oxide      | 0.10879 | 30.52     | 0.9961               | 23          | 0.9961              |            |
| (No. 4)    | 0.12485 | 34.97     | 0.9976               | 18.5        | 0.9967              |            |
|            | 0.12655 | 35.53     | 0.9952               | 18.5        | 0.9943              |            |
|            | 0.11899 | 33.41     | 0.9952               | 18.5        | 0.9943              |            |
|            |         |           |                      |             | Av. 0.9953          | -0.04      |
| Cuprous    | 0.12334 | 34.57     | 0.9969               | 20          | 0.9961              |            |
| Oxide      | 0.12387 | 34.68     | 0.9981               | 20          | 0.9973              |            |
| (No. 5)    |         |           |                      |             | Av. 0.9967          | +0.10      |
|            |         |           |                      |             | Av.                 | +0.03      |
| Solution 2 |         |           |                      |             |                     |            |
| Sodium     | 0.12415 | 36.55     |                      |             | 1.0139              |            |
| Oxalate    | 0.09965 | 29.30     |                      |             | 1.0152              |            |
|            | 0.06175 | 18.23     |                      |             | 1.0111              |            |
|            | 0.09585 | 28.20     |                      |             | 1.0146              |            |
|            | 0.08210 | 24.25     |                      |             | 1.0106              |            |
|            |         |           |                      |             | Av. 1.0131          |            |
| Cuprous    | 0.07560 | 20.85     |                      |             | 1.0131              |            |
| Oxide      | 0.21385 | 59.00     |                      |             | 1.0127              |            |
| (No. 6)    | 0.09290 | 25.75     |                      |             | 1.0080              |            |
|            | 0.12660 | 35.05     |                      |             | 1.0092              |            |
|            | 0.16770 | 46.25     |                      |             | 1.0131              |            |
|            |         |           |                      |             | Av. 1.0112          | -0.19      |
| Cuprous    | 0.21855 | 60.30     |                      |             | 1.0127              |            |
| Oxide      | 0.20980 | 57.75     |                      |             | 1.0141              |            |
| (No. 7)    | 0.16965 | 46.75     |                      |             | 1.0139              |            |
|            | 0.22650 | 62.45     |                      |             | 1.0134              |            |
|            |         |           |                      |             | Av. 1.0135          | +0.04      |
|            |         |           |                      |             | Av.                 | -0.07      |

10 cc. of the ferric ammonium sulfate solution was added, and the mixture was warmed and stirred until all particles were in solution. It was then diluted with about 100 cc. of warm water and titrated with the permanganate solution at a temperature around 60° C. The end point was determined electrometrically by a combination of the methods described by Goode<sup>1</sup> and Willard and Fenwick.<sup>2</sup>

The results are given in Table 1. The factors used in making the calculations were as follows: 1 cc. of 0.05 *N* permanganate is equivalent to 3.35 mg. of sodium oxalate, or 3.5785 mg. of cuprous oxide. The average difference in the factors obtained by the two methods of standardization is two parts in one thousand.

#### COPPER DETERMINED BY TITRATION AND ELECTROLYTICALLY

Values for copper determined by permanganate titration and electrolytically on the same samples are given in Table 2. Bureau of Standards

TABLE 2.—*Comparison of titration and electrolytic methods of determining cuprous oxide obtained by reduction with dextrose*

| TITRATION |       | ELECTROLYSIS | DIFFERENCE |          |
|-----------|-------|--------------|------------|----------|
| mg. Cu    |       | mg. Cu       | mg. Cu     | per cent |
| 6.63      |       | 6.83         | +0.20      |          |
| 7.06      |       | 7.10         | +0.04      |          |
| 7.62      |       | 7.30         | -0.32      |          |
| 10.18     |       | 10.44        | +0.26      |          |
| 11.98     |       | 12.10        | +0.12      |          |
| 12.29     |       | 12.20        | -0.09      |          |
| 20.03     |       | 20.29        | +0.26      |          |
| 20.53     |       | 20.80        | +0.27      |          |
| 25.85     |       | 26.39        | +0.44      |          |
| 26.93     |       | 27.21        | +0.28      |          |
| 30.97     |       | 31.39        | +0.42      |          |
| 31.44     |       | 31.81        | +0.37      |          |
| 33.66     |       | 33.95        | +0.29      |          |
| 34.38     |       | 34.73        | +0.35      |          |
| 38.91     |       | 38.82        | -0.09      |          |
| 47.45     |       | 47.61        | +0.16      |          |
| 47.95     |       | 48.41        | +0.46      |          |
| 61.58     |       | 61.92        | +0.34      |          |
| 72.97     |       | 73.49        | +0.52      |          |
| 86.63     |       | 86.41        | -0.22      |          |
| 88.67     |       | 88.61        | -0.06      |          |
| 99.24     |       | 99.70        | +0.46      |          |
| 112.97    |       | 112.86       | -0.11      |          |
| 113.46    |       | 112.89       | -0.57      |          |
| Av.       | 43.72 | 43.88        | +0.16      | +0.37    |

<sup>1</sup> *J. Am. Chem. Soc.*, **44**, 26 (1922).

<sup>2</sup> *Ibid.*, 2516.

glucose was used to precipitate the cuprous oxide according to the Quisumbing and Thomas method.<sup>1</sup> The cuprous oxide was dissolved in 10 cc. of the ferric ammonium sulfate solution and titrated with permanganate that had been standardized against sodium oxalate. The end point was determined electrometrically. When the titration was completed the solution was filtered free from asbestos and the copper was determined electrolytically.

The average difference obtained by the two methods is +0.16 mg. of copper.

Comparative results by the two methods obtained on the acid hydrolysate of phlorizin and the takadiastase hydrolysate of starch are given in Table 3.

TABLE 3.—*Comparison of permanganate and electrolytic methods of determining cuprous oxide prepared by reduction of the acid hydrolysate from phlorizin and of the takadiastase hydrolysate from starch*

| TITRATION | ELECTROLYSIS | DIFFERENCE | DIFFERENCE | TITRATION | ELECTROLYSIS | DIFFERENCE | DIFFERENCE |
|-----------|--------------|------------|------------|-----------|--------------|------------|------------|
| mg. Cu    | mg. Cu       | mg. Cu     | per cent   | mg. Cu    | mg. Cu       | mg. Cu     | per cent   |
| 16.87     | 16.80        | -0.07      |            | 12.19     | 12.3         | +0.11      |            |
| 16.99     | 17.05        | +0.06      |            | 14.19     | 14.5         | +0.31      |            |
| 16.96     | 17.01        | +0.05      |            | 14.57     | 14.4         | -0.17      |            |
| 16.81     | 16.86        | +0.05      |            | 15.77     | 16.1         | +0.33      |            |
| 17.90     | 17.83        | -0.07      |            | 26.28     | 26.4         | +0.12      |            |
| 17.49     | 17.83        | +0.34      |            | 25.80     | 25.5         | -0.30      |            |
| 18.05     | 17.92        | -0.13      |            | 26.76     | 26.9         | +0.14      |            |
|           |              |            |            | 27.48     | 27.9         | +0.42      |            |
|           |              |            |            | 54.24     | 54.9         | +0.66      |            |
|           |              |            |            | 55.67     | 55.6         | -0.07      |            |
| Av. 17.30 | 17.33        | +0.03      | +0.17      | 27.30     | 27.45        | +0.15      | +0.55      |

The results are in good agreement although the electrolytic method gives results which are slightly higher than those given by the titration method. However, the differences, even if significant, are too small to be of any practical importance in the estimation of reducing sugars.

For the electrolytic determination following the permanganate titration, the solution was filtered off and acidified with concentrated nitric acid. Hydrogen peroxide was then added, and electrolysis was continued until all the copper was deposited on the electrode. It was found necessary to continue electrolysis longer than is the case when manganese and iron are not present. The accuracy of the method was tested with known amounts of copper sulfate to which manganese and iron were added in amounts similar to those present in the case of the determination of

<sup>1</sup> *J. Am. Chem. Soc.*, 43, 1503 (1921).

reducing sugars. The data in Table 4 show that the method used is accurate in determining the copper in the presence of manganese and iron.

TABLE 4.—*Determination of copper electrolytically in  $\text{CuSO}_4$  in the presence of manganese and iron*

| SOLUTION | COPPER SULFATE ALONE | COPPER SULFATE + MANGANESE AND IRON |
|----------|----------------------|-------------------------------------|
|          | mg. Cu               | mg. Cu                              |
| 1        | 120.17               | 120.16                              |
| 1        | 120.31               | 120.05                              |
| 2        | 33.09                | 33.16                               |
| 2        | 33.14                | 33.12                               |

### DISCUSSION

In the first attempts made to standardize the permanganate solution higher values were obtained with cuprous oxide than with sodium oxalate. It was found that this was due to the fact that the dry cuprous oxide was not entirely dissolved in the ferric ammonium sulfate. When precautions were taken to insure complete solution of the cuprous oxide the values obtained by the two methods showed close agreement.

It is believed that the erroneous statements in the literature to the effect that two low results are obtained when the permanganate is standardized with sodium oxalate are due to the fact that it was not recognized that standardization with cuprous oxide may yield too high results if precautions are not taken to insure complete solution of the cuprous oxide in the standardization of the permanganate.

On the other hand, if the permanganate is standardized accurately the results of the determination of reduced copper by the permanganate method will be too low unless extreme care is taken to insure that the cuprous oxide is completely dissolved in the ferric ammonium sulfate.

Best results were obtained by transferring the asbestos mat containing the  $\text{Cu}_2\text{O}$  to the beaker with a stirring rod and then adding the ferric ammonium sulfate and stirring the asbestos thoroughly to permit solution of the  $\text{Cu}_2\text{O}$  before adding warm water. The solution was then stirred vigorously during the titration period with a motor driven stirrer in order to insure complete solution of the cuprous oxide. Another source of error, inaccuracy in the determination of the end point, may be avoided by using an electrometric method for determining the end point.

### SUMMARY

1. Standardization of the permanganate solution for the estimation of reducing sugars with cuprous oxide or sodium oxalate yields values in close agreement. Too high values will be obtained with cuprous oxide if extreme care is not taken to insure complete solution of the cuprous oxide.

2. To determine accurately the reduced copper by means of the per-



manganate titration method it is necessary to secure complete solution of the cuprous oxide in the ferric ammonium sulfate and to obtain the correct end point of the titration.

3. The standardization of the permanganate solution with sodium oxalate does not give results that are too low in the estimation of reducing sugars, as is reported in the literature.

4. When permanganate that was standardized against sodium oxalate was used in the determination of reduced copper in the estimation of reducing sugars, values in close agreement with those obtained by the electrolytic method resulted.

## DITHIZONE METHODS FOR THE DETERMINATION OF LEAD

By P. A. CLIFFORD and H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.)

Since its introduction by H. Fischer<sup>1</sup> in 1929, the reagent dithizone has been extensively applied to the determination of small quantities of lead, and following the original Fischer and Leopoldi<sup>2</sup> publication on the colorimetric determination of lead numerous modifications appeared. In this paper the writers classify these various methods and discuss their advantages and disadvantages. The details of a "mixed color" method, which utilizes to a greater extent the remarkable sensitivity of this reagent, are also given.

Dithizone has the formula  $C_6H_5NHNHCSN=NC_6H_5$ , and according to Fischer,<sup>3</sup> it exists in both enol and keto forms. It crystallizes from chloroform in the form of purplish-black rods and needles, readily soluble in chloroform and to a lesser extent in carbon tetrachloride. Its solubility in water or dilute mineral acids is negligible, but it is very soluble in dilute ammonia, presumably going into solution as the ammonium salt of the enolic modification. Although commercial dithizone is now of much better quality than it was formerly, for accurate colorimetric work purification is still necessary. This is readily done, as the usual impurities (sulfur, oxidation products of dithizone, and varying amounts of the intermediate carbazide) are not soluble in ammonia. The method for purification given previously<sup>4</sup> may be employed with the further precaution that all reagents used must be redistilled and metal-free. A practical test of purity is the extraction of a strong chloroform solution of the reagent with dilute metal-free ammonia and observation of the final color of the chloroform layer. If it "strips" water-white, the dithizone is pure. Light yellow to brown colors in the chloroform phase indicate the necessity

<sup>1</sup> *Angew. Chem.*, 42, 1025 (1929).

<sup>2</sup> *Wiss. Veröffentlich. Siemens-Konzern*, 12, 44 (1933).

<sup>3</sup> *Angew. Chem.*, 47, 685 (1934).

<sup>4</sup> *This Journal*, 17, 117 (1934).

for purification. The pure reagent should keep for weeks in chloroform or carbon tetrachloride solution, provided these solvents are free from oxidizing substances. They should always be carefully redistilled before use and discarded if they yield a positive test for chloride.

The reagent has great tinctorial power as 1 part in 40,000,000 parts of chloroform gives a noticeable green tint. Dilute solutions of dithizone in chloroform or carbon tetrachloride appear blue-green or grass-green, respectively, by transmitted light. When the concentration or depth of column is increased sufficiently, the color of the transmitted light changes abruptly from green to red. This change affords a preliminary estimation of the strength of dithizone solutions. The depth of column (mm.) of a chloroform solution of dithizone that just begins to impart a red color to transmitted light, multiplied by the concentration expressed in mg. per liter, is approximately equal to the constant 380. With carbon tetrachloride the color change is quite as abrupt, but it requires about 2.5 times the concentration or depth of column. Transmission spectra of even dilute chloroform or carbon tetrachloride solutions of dithizone show a strong absorption band centering at about 600  $m\mu$  with a weaker one at about 450  $m\mu$  (see Fig. 1). As concentration increases these absorption bands widen until eventually the transmitted light is predominantly red. The change in appearance of the absorption bands with increasing concentration is illustrated by the spectrograms of Plate I.

In one or the other of its modifications dithizone dissolved in chloroform or carbon tetrachloride will react under varying conditions with at least 14 different metals. The resulting complexes are likewise usually soluble in chloroform and can be extracted into the nonaqueous phase. These complexes are always colored and range from purple through yellow and orange to red. The reagent is therefore by no means specific, but by previous fixation of interferences as non-ionizing complexes it can be made nearly so. Thus, while dithizone will react in a citrated alkaline solution with a number of metals, among them zinc, cobalt, nickel, cadmium, silver, copper, mercury, stannous tin, bismuth, thallium, and lead, the presence of excess potassium cyanide will prevent reaction with all but the last four. In other words, stannous tin, bismuth, and thallium are the interferences to be guarded against in the determination of lead.

The color of the lead-dithizone complex in chloroform or carbon tetrachloride solution is a cherry-red, and hence the progress of extraction can be followed by noting the point at which additional portions of dithizone solution retain their normal green color. The writers<sup>1</sup> and others<sup>2,3,4</sup> have made extended use of this extraction process for the isolation of lead preparatory to its electrolytic or other form of determination. As a solvent

<sup>1</sup> *This Journal*, 17, 118, 124 (1934); 18, 315 (1935).

<sup>2</sup> Allport and Schrimshire, *Analyst*, 57, 440 (1932).

<sup>3</sup> Lynch, Slater, and Oiler, *Analyst*, 59, 787 (1934).

<sup>4</sup> Wilkens, Willoughby, et al, *Ind. Eng. Chem. Anal. Ed.*, 7, 33 (1935).

chloroform is preferred to carbon tetrachloride because the solubility of the complex in the latter is decidedly limited, and if the quantity of the lead complex present exceeds this solubility, it will appear as a red sludge at the interface in the separatory funnel and make quantitative separation difficult.

According to Fischer<sup>1</sup> two molecules of dithizone react with one atom of lead. Therefore, for brevity the complex will be designated as  $PbD_2$ . It is well known that  $PbD_2$  dissolved in an immiscible solvent is readily decomposed by shaking the solution with dilute acid. The writers have shown by the following experiments that the complex, without excess lead or dithizone, is not stable unless the aqueous solution has a  $pH$  above 10.

A solution of pure dithizone in chloroform was nearly saturated with

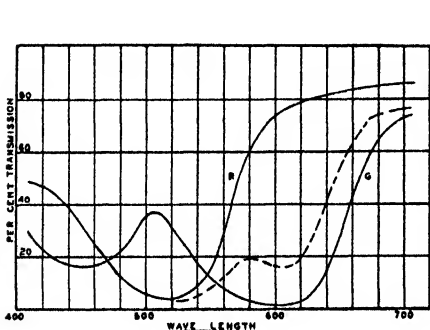


FIG. 1

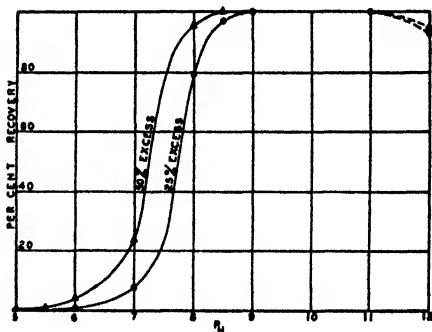


Fig. 2

FIG. 1.—CURVE “G”—TRANSMISSION THROUGH A ONE-INCH CELL OF AN APPROXIMATELY 5 MG./L. SOLUTION OF DITHIZONE IN CHLOROFORM  
CURVE “R”—TRANSMISSION OF THE SAME SOLUTION AFTER BEING SATURATED WITH LEAD.

FIG. 2.—RECOVERY OF LEAD AT VARIOUS  $pH$  WITH VARYING AMOUNTS OF EXCESS DITHIZONE (PER CENT).

lead, and the excess dithizone was removed by repeated extractions with dilute ammonia-potassium cyanide solution; 25 cc. portions of this chloroform solution of  $PbD_2$  were then shaken with the same volume of different buffer mixtures at about  $pH$  7.0, 8.0, 9.0, 10.0, 11.0, and 12.0. The color of the chloroform solutions changed from green at  $pH$  7.0 to a cherry-red at  $pH$  10.0, after which the change was slight. (“Buffer mixtures” at  $pH$  7.0, 8.0, 9.0, 10.0, and 11.0 were ammonia-potassium cyanide solutions adjusted with lead-free citric acid and checked colorimetrically with appropriate indicators: bromthymol blue, phenol red, thymol blue, thymolphthalein, alizarine yellow, and Poirrier’s blue, against stand-

<sup>1</sup> *Loc. cit.*



PLATE I

1. Light source through cell and solvent.
2. 10 mg /l. dithizone in chloroform. Visual color green
3. 15 mg./l. dithizone in chloroform. Visual color intermediate.
4. 25 mg /l. dithizone in chloroform. Visual color red.
5. 25 mg /l. dithizone in carbon tetrachloride. Visual color green.
6. 25 mg /l. dithizone in dilute ammonia (1+200). Visual color orange.
7. 40 mg./l. dithizone in carbon tetrachloride. Visual color intermediate.



PLATE II

1. Light source through cell and solvent.
2. Dithizone solution in chloroform about 5 mg./l.
3. Dithizone solution in chloroform 20% saturated with lead.
4. Dithizone solution in chloroform 40% saturated with lead.
5. Dithizone solution in chloroform 60% saturated with lead.
6. Dithizone solution in chloroform 80% saturated with lead.
7. Dithizone solution in chloroform 100% saturated with lead.

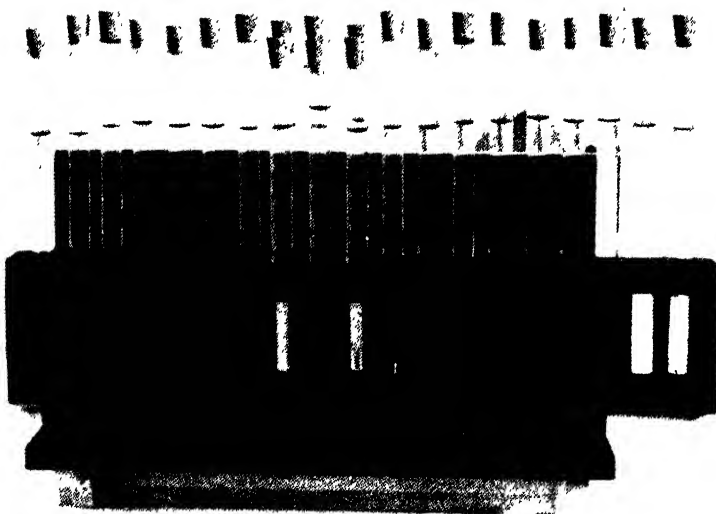


PLATE III

Large comparator box for the determination of lead in spray residue by the original Vorhes-Clifford technic.

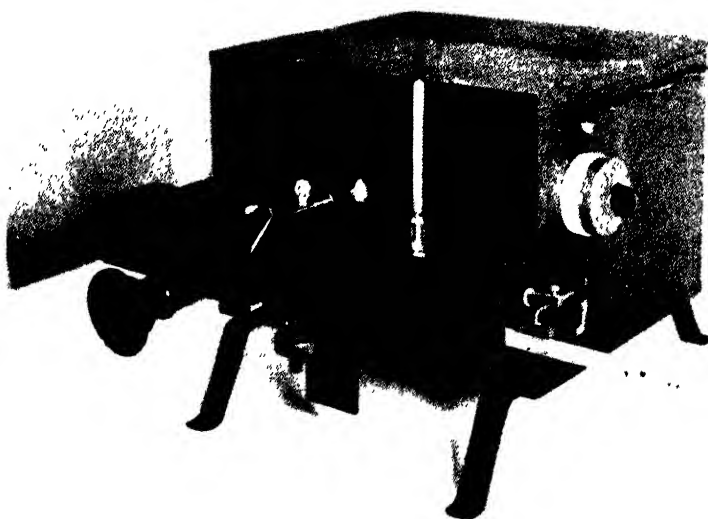


PLATE IV

Neutral wedge photometer.

ard buffer solutions. At pH 12.0 sodium hydroxide was substituted for ammonia.)

The color changes between pH 10.0 and 7.0 are due to a slight to nearly complete dissociation of the lead complex, whereby the liberated dithizone reverts to the green form and modifies the color of the chloroform layer. An equivalent amount of lead appears in solution in the aqueous phase. (Above pH 11.0 lead drawn into the ammoniacal aqueous phase is slowly precipitated.) The proportion of uncombined lead at the various pH levels was determined as follows: A solution of  $PbD_2$  in chloroform was prepared as above and analyzed for lead content by a photometric method described later; 25 cc. portions were then shaken out as before at various pH levels. The chloroform fractions were discarded, the aqueous portions were centrifuged, and the lead in 20 cc. aliquots was determined by the "mixed color" method described later. The lead found in the aqueous portion is given in Table 1 as percentage of the total amount originally present as  $PbD_2$ .

TABLE 1.—*Relative stability of  $PbD_2$  at varying pH*

| pH                     | 7.0  | 8.0  | 9.0  | 10.0 | 11.0    |
|------------------------|------|------|------|------|---------|
| % in aqueous phase (1) | 96.0 | 65.0 | 17.6 | 3.1  | About 1 |
| % in aqueous phase (2) | 99.0 | 75.3 | 36.8 | 9.6  | 3.2     |

(1) Total amount of Pb as  $PbD_2$  in 25 cc. of  $CHCl_3$  = 31.8 gamma  
 (2) Total amount of Pb as  $PbD_2$  in 25 cc. of  $CHCl_3$  = 15.6 gamma

These observations show that the reaction between lead and dithizone is a reversible one and that definite equilibria exist at various pH levels, even above neutrality. This equilibrium at definite pH can be shifted by adding either of the reactants in excess. For example, a chloroform solution of pure  $PbD_2$  after being shaken with a buffer mixture at pH 8.0 has a bluish-purple color. The addition of a small crystal of lead nitrate and further shaking change the color to the fully developed red. It appears to be a simple mass-action effect. Furthermore, lead can be forced into the chloroform phase and extracted as  $PbD_2$  by shaking with an excess of dithizone at pH levels below 10 or 11. However, a considerable excess of dithizone and several extractions may be necessary in order to remove lead quantitatively from aqueous solutions with a pH barely above the neutral point.

The data in Table 2 give a rough idea of the efficiency of extraction with different amounts of excess dithizone, at various pH levels. In these experiments 0.5 mg. of lead was added to a series of 100 cc. volumetric flasks, and after the addition of a few cc. of ammonia-potassium cyanide solution, the mixtures were adjusted to approximately the pH shown with lead-free citric acid. After being made to volume, aliquots of 50 cc.

were extracted with *one* 50 cc. portion of dithizone in chloroform containing either 15.5 or 18.6 mg. per liter, that is, enough to furnish, respectively, a 25 or a 50 per cent excess of dithizone. The extracted lead was determined electrolytically<sup>1</sup> if the amount was above 0.1 mg., or by the sensitive "mixed-color" method later described for amounts below this figure.

TABLE 2.—*Recovery of lead with excess dithizone at varying pH*

| pH   | 25% EXCESS   |          | 50% EXCESS   |          |
|------|--------------|----------|--------------|----------|
|      | Pb RECOVERED |          | Pb RECOVERED |          |
|      | mg.          | per cent | mg.          | per cent |
| 5.0  |              |          | 0.0004       | 0.16     |
| 5.5  |              |          | 0.0015       | 0.60     |
| 6.0  | 0.0016       | 0.64     | 0.0099       | 4.0      |
| 7.0  | 0.019        | 7.6      | 0.059        | 23.6     |
| 8.0  | 0.198        | 79.      | 0.239        | 96.      |
| 8.5  | 0.242        | 97.      | 0.249        | 100.     |
| 9.0  | 0.249        | 100.     |              |          |
| 11.0 | 0.249        | 100.     | 0.248        | 99.      |
| 12.0 | 0.233        | 93.      | 0.239        | 95.      |

When plotted, these data give the interesting curves shown in Fig. 2. It is seen that a 25 per cent excess of dithizone in one extraction under the conditions of the experiment completely extracts the lead at pH 9.0, where the pure complex, without excess lead or dithizone, is normally about 25 per cent dissociated. Larger amounts of excess dithizone completely extract lead at lower pH and extend the pH range at which measurable amounts of lead are extracted to considerably below the neutral point. The dotted lines between pH 11 and 12 are intended to indicate a secondary dissociation, whereby an indeterminate amount of lead (depending largely upon time of shaking) is drawn once more into the aqueous phase. This amount depends, as before, upon the amount of excess dithizone. With carbon tetrachloride as solvent this loss would be much greater.

A series of similar curves would show graphically the conditions governing the extraction of lead from aqueous solution. A real danger of lead loss occurs if the solution is extracted at too low a pH with too weak a dithizone solution. In actual lead determinations solutions of ashed materials may be strongly buffered near the neutral point when ammonia is used for neutralization. In such cases it may not be safe to depend upon litmus paper as indicator, and the writers urge the use of thymol blue (8.2–9.8) for more positive pH control. The appearance of uncombined dithizone in the extract does not necessarily indicate complete extraction of lead, and unless the operator is sure that the pH is above

<sup>1</sup> Wichmann and Clifford, *This Journal*, 18, 315 (1935).

a safe level, repeated extraction with excess dithizone is advisable. There is also the danger of possible lead loss by dissociation, after extraction from ammoniacal solution with a bare excess of dithizone, if the extracts are washed with water preparatory to electrolytic (or other) determination. If washing is required, a drop or two of ammonia in the wash water will raise the  $pH$  to a safe level, but excess of ammonia is to be avoided for the reasons outlined previously.

Similar recovery curves for various metals would also show the possibility of quantitative separation by extraction with dithizone *under controlled conditions of  $pH$* . Thus, Willoughby et al.<sup>1</sup> separate bismuth from lead by extracting with a large excess of dithizone at  $pH$  2.0. While this low  $pH$  is certainly not optimum for the bismuth-dithizone reaction, repeated extraction with excess dithizone permits the removal of considerable amounts, and the quantity of lead lost is negligible. On the other hand, Fischer,<sup>2</sup> Winter et al.<sup>3</sup> and Tompsett and Anderson<sup>4</sup> attempt separation of bismuth from lead by washing the combined dithizone extracts with dilute potassium cyanide or ammonia-potassium cyanide solutions. This procedure will certainly cause some loss of lead and a possible compensation of errors, as Fig. 2 clearly shows loss of lead in aqueous solutions of high  $pH$ . However, these considerations do indicate that the shape of the bismuth recovery curves would probably be very similar to those for lead with the exception of being displaced 2 or 3  $pH$  units to the left. Dissociation curves for bismuth and the other dithizone metals would be very useful in devising dithizone methods for their determination.

These recovery experiments open the way to a series of interesting equilibrium studies on the two-phase lead-dithizone system. It is certain that the equilibrium ratio between lead in combination in the chloroform phase and dissociated lead in the aqueous phase is definite, at fixed  $pH$ , for any given amount of dithizone, and it should be possible to formulate a universal equilibrium constant for the reaction by including the  $pH$  variable. Thus efficiency of extraction under all conditions could be calculated. This would necessitate the study of the distribution of dithizone between chloroform and aqueous solutions of various  $pH$  and recovery experiments under more careful  $pH$  control than has been attempted here.

#### PRINCIPLES OF METHODS

The various dithizone methods that have been proposed by the writers or by others may be classified as (1) titrimetric-extraction methods, (2) "one-color" methods, and (3) "mixed-color" methods. The principles of these methods will be discussed briefly.

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<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, **7**, 285 (1935).

<sup>2</sup> *Angew. Chem.*, **47**, 90, 685 (1934).

<sup>3</sup> *Ind. Eng. Chem. Anal. Ed.*, **7**, 265 (1935).

<sup>4</sup> *Biochem. J.*, **29**, 1851 (1935).



## TITRIMETRIC-EXTRACTION METHODS

These methods involve the extraction of lead from aqueous solution at definite pH with successive increments of standardized dithizone solution until the green dithizone is no longer changed to red at the end point. The "titration" is made in a separatory funnel with sufficient shaking between additions to establish equilibrium between lead and dithizone, and the solvent layer containing the red lead complex is drawn off from time to time. The dithizone solution is standardized against known amounts of lead in the same manner.

This principle has been applied by Bohnenkamp & Linneweh<sup>1</sup> to the determination of lead; by H. Fischer<sup>2</sup> to silver, zinc, and cadmium; and by Winkler<sup>3</sup> to mercury. Wilkins, Willoughby, et al.<sup>4</sup> isolate lead with dithizone from digested blood, urine, etc., then retransfer the lead to the aqueous phase by shaking the chloroform solution with 1 per cent nitric acid. They then adjust the pH of the lead solution to 7.5 (phenol red) and titrate with standardized dithizone solution as described previously. Their results are claimed to be accurate and sensitive to 0.001 mg. of lead.

While sound in principle, titrimetric-extraction methods are tedious, and the end point may be overstepped if the dithizone solution is added in too large portions. However, it is possible to make a preliminary rough extraction, reserve the extracts, and retransfer the lead to the aqueous phase as directed above. The titration is then repeated, and the end point is approached more slowly.

The titration should be conducted at a pH of relative stability of  $PbD_2$ . On this point the method of Wilkins and Willoughby seems at fault, because previous data (Table 1) show the lead complex to be largely dissociated at pH 7.5. At the beginning of a titration the lead is in excess, and the resulting color in the solvent phase is red. However, as the lead is progressively removed from the aqueous phase, its mass-action effect is lost, and toward the end of the extraction intermediate hues of purple develop. R. U. Bonnar<sup>5</sup> reports that it requires considerably more than the equivalent amount of dithizone to reach an end point at pH 7.5. At higher pH the dissociation is repressed, and the end point from red to green is much more abrupt. Less shaking is likewise required for establishing equilibrium. However, too high a pH encourages emulsion formation. At pH 11.0 or over, the  $PbD_2$  in the absence of excess dithizone becomes appreciably soluble in the aqueous phase, and the end point again loses sensitivity. The writers believe a pH of about 9.5 (incipient blue with thymolphthalein) is about optimum for titrimetric work.

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<sup>1</sup> *Deut. Arch. klin. Med.*, 175, 157 (1933).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *This Journal*, 18, 638 (1935).

<sup>4</sup> *Loc. cit.*

<sup>5</sup> U. S. Department of Agriculture. Private communication.

#### "ONE COLOR" METHODS

In these methods the lead is extracted with a small excess of dithizone in chloroform or carbon tetrachloride solution, and the excess dithizone is removed from the combined extracts by washing with dilute ammonia, potassium cyanide solution, or mixtures of the two. The amount of lead in the extract is then estimated colorimetrically in one of two ways: (1) The washed extract may be shaken out with dilute acid and the reverted green color of the equivalent amount of dithizone measured by suitable means; or (2) the red color of the  $PbD_2$  may be measured directly. In either case the color produced is compared with that obtained when known quantities of lead are carried through the same procedure. In the original "one-color" method Fischer & Leopoldi<sup>1</sup> extracted excess dithizone from the carbon tetrachloride solution of  $PbD_2$  with 5 cc. portions of ammonia (1+200) and, using procedure (1), measured the color of the green phase with a Hellige wedge colorimeter. Their data show the determination of 0.01–0.120 mg. of lead with an error of 0.001–0.003 mg., or 3–8 per cent.

Seelkopf and Taeger<sup>2</sup> use the Fischer method for the removal of excess dithizone and, using procedure (2), determine the extinction coefficient of the red phase with a Zeiss-Pulfrich photometer and monochromatic green filter (530  $m\mu$ ). Their standard curve of known concentrations of lead plotted against extinction coefficients is linear. Their error on 0.005–0.020 mg. of lead is 1–10 per cent, depending on the amount of lead present.

Winter et al.<sup>3</sup> use chloroform instead of carbon tetrachloride as the solvent, strip with a more alkaline solution, ammonia (1+100) plus 0.1 per cent potassium cyanide, and match the colors in an ordinary colorimeter against a standard solution set at definite depth. The reference curve obtained has a decidedly hyperbolic form. They have difficulty in the stripping operation due to the greater solubility of dithizone in chloroform. The sensitivity of their method is reported as about 0.001 mg. Ross and Lucas<sup>4,5</sup> and Tompsett and Anderson<sup>6</sup> employ essentially the same procedure for the determination of lead in urine.

These writers assume (1) complete extraction of uncombined dithizone from the chloroform or carbon tetrachloride solutions of the lead extracts, and (2) negligible solubility of  $PbD_2$  in the dilute ammonia used in the stripping operations. These assumptions are not strictly true, and therefore the stripping technic of "one-color" methods results in a small compensation of errors. The same compensation must be made in the standards for accurate results.

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Z. exp. Med.*, 91, 359 (1933).

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *J. Biol. Chem.*, 111, 2 (1935).

<sup>5</sup> *Canadian Med. Assoc. J.*, 29, 649 (1933).

<sup>6</sup> *Loc. cit.*

(1) *Extraction of Excess Dithizone.*—The excess dithizone remaining after extraction of the lead partitions between the nonaqueous solvent and dilute ammonia phases according to a definite distribution ratio and is theoretically never completely removed. Any dithizone retained in the solvent will cause a positive error in results.

Distribution ratios were determined for the partition of dithizone between carbon tetrachloride and ammonia of various strengths as follows: A solution of carefully purified dithizone in carbon tetrachloride was made up by direct weight. The absorption of red light by various dilutions of the solution was measured photometrically by means of a tungsten light source and suitable red filter. The curve plotted from these data, while not entirely linear, provided the means for the estimation of the concentration of subsequent solutions of dithizone.

Portions of 25 cc. of the carbon tetrachloride solutions of dithizone were then shaken for 3 minutes with 5 cc. portions of ammonia (1+200). (The ammonia was redistilled and carefully adjusted to 1+200 strength in CO<sub>2</sub>-free redistilled water by titration, and to minimize volume changes the dithizone solutions were saturated with water before being extracted with the ammonia.) The amounts of dithizone passing into the ammonia phase were determined by drawing off the carbon tetrachloride fraction, acidifying the aqueous portion slightly, and re-extracting with 25 cc. of clear carbon tetrachloride. These solutions were then run through lead-free filters and read in the photometer. The difference between these readings and the original readings of the carbon tetrachloride solutions was taken as the amount of dithizone remaining in the carbon tetrachloride at equilibrium. These amounts were likewise determined directly, but as the solutions were comparatively weak after extraction, the experimental error was considered to be proportionately greater. The direct values are given for reference. These results are summarized in Table 3.

TABLE 3.—*Partition of dithizone between 25 cc. of CCl<sub>4</sub> and 5 cc. of NH<sub>4</sub>OH (1+200)*

|  | (Amounts of dithizone in gamma. Temp. = 31°C.) |      |      |      |      |      |      |      |
|--|--|------|------|------|------|------|------|------|
| Dithizone taken                              | 615  | 500  | 320  | 167  | 101  | 67.5 | 36.8 | 18.8 |
| Dithizone ext'd.                             | 552  | 451  | 289  | 145  | 91.8 | 60.1 | 32.0 | 16.0 |
| Residual by diff.                            | 63   | 49   | 31   | 22   | 9.2  | 7.4  | 4.0  | 2.8  |
| Residual (direct)                            | 67   | 45   | 30   | 15   | 9.3  | 6.5  | 2.6  | 1.2  |
| Extracted (%)                                | 90   | 90   | 90   | 87   | 91   | 89   | 89   | 85   |
| $\frac{\text{CNH}_4\text{OH}}{\text{CCl}_4}$ | 43.8   | 46.0 | 46.6 | 33.0 | 49.9 | 40.6 | 40.0 | 28.6 |
| Av. distribution coefficient = 41.1          |  |      |      |      |      |      |      |      |

When carbon tetrachloride is used as the solvent, the quantity of dithizone retained after the stripping operation can be calculated, provided the amount of excess remaining in the original extract is known.

This amount will vary within wide limits, depending upon the amount of lead to be extracted and, above all, upon the alkalinity and volume of the original aqueous solution. Using H. Fischer's original technic, the writers determined that occasionally as much as 0.1 mg. of excess dithizone was present in the original carbon tetrachloride extracts, although the average figure was 0.03–0.04 mg. Hence it can be shown that it is possible to retain, roughly, 0.2–1.0 gamma of dithizone in the carbon tetrachloride solution, the amount depending upon its volume, even after two strippings with 5 cc. portions of ammonia (1+200).

The extent of the positive error caused by incomplete removal of excess dithizone depends upon the method used for the estimation of lead. If Fischer's method of decomposing  $PbD_2$  and measuring the green phase is employed, the error is a direct one; if the red phase is measured with a photometer and monochromatic green filter (Seelkopf and Taeger), the error is less, as dilute dithizone solutions in carbon tetrachloride transmit freely in the green.

The greater solubility of dithizone in chloroform is illustrated by the much smaller distribution ratio derived from the following set of data. Here, likewise, a standard curve was prepared to give concentration of chloroform solutions against absorption coefficient. A green filter centering at about  $520m\mu$  was used in the photometer, as the transmission of even moderately concentrated (20 mg./l.) solutions of dithizone in chloroform through a 1 inch cell is greater in the red than in the green. The curve obtained was nearly linear (Fig. 3). The technic of the partition experiments was the same as that with carbon tetrachloride. Results are given in Table 4.

TABLE 4.—Partition of dithizone between 20 cc. of  $CHCl_3$  and 10 cc. of  $NH_4OH$  (1+200)

(Amounts of dithizone in gamma. Temp. = 30° C.)

|                      |                                     |      |      |      |      |
|----------------------|-------------------------------------|------|------|------|------|
| Dithizone taken      | 264                                 | 168  | 76.4 | 46.2 | 17.8 |
| Dithizone ext'd.     | 123                                 | 77   | 34.8 | 20.0 | 7.6  |
| Residual by diff.    | 141                                 | 91   | 41.6 | 26.2 | 10.2 |
| Residual (direct)    | 147                                 | 94   | 41.2 | 27.0 | 11.0 |
| Extracted (per cent) | 47                                  | 46   | 46   | 43   | 43   |
| $C_{NH_4OH}$         | 1.74                                | 1.69 | 1.67 | 1.53 | 1.49 |
| $C_{CHCl_3}$         | Av. distribution coefficient = 1.62 |      |      |      |      |

The stripping mixture used by Winter et al.<sup>1</sup> was ammonia (1+100) made 0.1 per cent with potassium cyanide. The partition ratio of dithizone between this reagent and chloroform was found to be about 2.5. Although these investigators were aware of the possibility of incomplete

<sup>1</sup> *Loc. cit.*

stripping, it is likely that small amounts of free dithizone may have remained in their chloroform extracts.

The solubility of dithizone in aqueous solution decreases as the alkalinity is lowered and the partition ratios, as expressed above, diminish. Thus, at pH 10 the ratio for carbon tetrachloride was found to be 19.4 and that for chloroform was 0.76. Winter et al. noticed that the addition of ammonium citrate decreased the solubility of dithizone in dilute ammoniacal solution. This is because the pH was lowered.

(2) *Solubility of PbD<sub>2</sub> in Solutions of High pH.*—There is a readily detectable loss of lead when carbon tetrachloride or chloroform solutions of PbD<sub>2</sub> are shaken with the dilute ammonia or ammonia-potassium cyanide solutions used by the investigators cited above in the stripping operation. The extent of this loss depends upon several factors. Lead is more readily drawn from carbon tetrachloride than from chloroform solutions of PbD<sub>2</sub>. Time of shaking is another factor because the lead drawn into the aqueous phase is slowly precipitated. Hence, there is no definite partition, and prolonged shaking results in increased loss. Decomposition of the complex and resultant lead loss is repressed by the presence of free dithizone, and finally, loss of lead increases as the pH of the stripping solution is raised. These effects were investigated by the following procedure:

A solution of pure PbD<sub>2</sub> in carbon tetrachloride was prepared by nearly saturating a solution of dithizone in this solvent with lead and removing the excess dithizone by repeated stripping with dilute ammonia. The solution was then filtered, and the lead content of measured portions was determined electrolytically. By the use of a monochromatic green filter (520m $\mu$ ) and photometer, absorption coefficients of various dilutions of this solution were determined, and by plotting absorption coefficient against concentration of lead a curve was obtained from which the lead content of any carbon tetrachloride solution of PbD<sub>2</sub> could be estimated. A curve for lead as PbD<sub>2</sub> in chloroform was obtained in a similar way. The two curves were linear and nearly identical (Fig. 4). Thus when portions of the PbD<sub>2</sub> solutions of various concentration were shaken out with the stripping reagents, the lead loss could be readily determined from the absorption measurements before and after shaking.

A 25 cc. portion of PbD<sub>2</sub> in carbon tetrachloride containing 35.8 gamma of lead was shaken one minute with 25 cc. of ammonia (1+200). Enough of the clear lower layer was drawn off to fill the cell of the photometer, and the photometric readings of the solution before and after shaking indicated a loss of 6.4 gamma of lead. The remainder of the mixture in the funnel was drawn off into a vial, and the aqueous portion was clarified by means of the centrifuge. An analysis of a 20 cc. aliquot of the aqueous portion (withdrawn by means of a pipet) showed only 1.4 gamma of lead in actual solution. The remaining contents of the vial were run

through a fritted glass filter, the separatory funnel and vial were flushed out, and the filter was thoroughly washed with clear carbon tetrachloride. The filter was then rinsed with dilute lead-free nitric acid, and 3.7 gamma of lead was recovered, showing that the major part of the lead withdrawn from the solvent phase precipitates out and can be recovered by filtration. When shaking was prolonged for 10 minutes, the indicated loss was 10.8 gamma, of which 6.3 gamma was recoverable by filtration. If a  $PbD_2$  solution of sufficient strength is shaken with ammonia (1+200) the precipitated particles can readily be seen with the microscope.

The presence of free dithizone reduces the lead loss in the stripping operation. Thus, when 30 cc. of a carbon tetrachloride solution of  $PbD_2$  was shaken one minute with 25 cc. of ammonia (1+200), the lead loss was 6.1 gamma. When the 30 cc. of carbon tetrachloride contained in

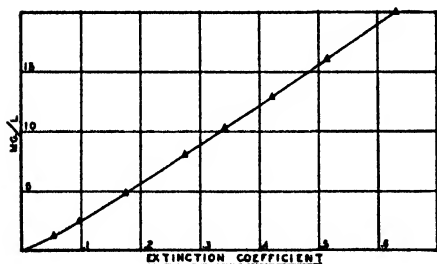


FIG. 3

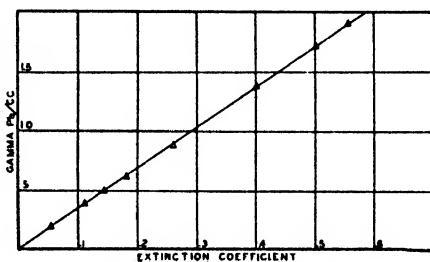


FIG. 4

FIG. 3.—EXTINCTION COEFFICIENT—CONCENTRATION RELATIONSHIP FOR DITHIZONE IN CHLOROFORM OBTAINED WITH A MONOCHROMATIC (520  $m\mu$ ) FILTER.

FIG. 4.—EXTINCTION COEFFICIENT—CONCENTRATION RELATIONSHIP FOR  $PbD_2$  IN CHLOROFORM OBTAINED WITH A MONOCHROMATIC (520  $m\mu$ ) FILTER.

addition 10 gamma of free dithizone, the loss (determined by the "mixed-color" method) was 3.9 gamma, and when 50 gamma of free dithizone was present, the loss was only 0.7 gamma.

TABLE 5.—Lead loss from carbon tetrachloride solutions in the stripping operation  
(Amounts of lead in gamma)

|                               |      |      |      |      |     |
|-------------------------------|------|------|------|------|-----|
| Lead content before stripping | 31.8 | 25.3 | 20.4 | 14.8 | 9.5 |
| Lead content after stripping  | 31.0 | 24.7 | 19.7 | 14.0 | 8.8 |
| Lead loss                     | .8   | .6   | .7   | .8   | .7  |

The results (Table 5) show the lead loss that might be expected when Fischer's technic of stripping with 5 cc. of ammonia (1+200) is used. The volume of the carbon tetrachloride fractions was 25 cc., and the mixtures were shaken 30 seconds.

The same general considerations govern the loss of lead from chloroform solutions of  $PbD_2$ . The lesser tendency toward loss in the stripping process is outweighed by the fact that larger volumes of a more alkaline stripping agent are necessary to extract efficiently excess dithizone. When the Winter stripping mixture (1+100 ammonia plus 0.1 per cent potassium cyanide) was used, lead losses were 1.8, 1.4, and 1.2 gamma when 25 cc. portions of chloroform solutions of  $PbD_2$  containing, respectively, 31.3, 22.3, and 13.8, were shaken for 30 seconds with 20 cc. of the above stripping mixture. With 1+200 ammonia the losses ranged from 0.7 to 0.3 gamma for comparable amounts of lead.

Thus it is difficult to evaluate the negative error of "one-color" methods. If there is an excess of uncombined dithizone in the solvent phase, the loss of lead in the stripping process is slight, but as this excess is removed the loss at pH 11 and above becomes appreciable. Washing at a lower pH will not help, because the excess dithizone is then removed with greater difficulty, and losses of lead due to slight dissociation of  $PbD_2$  will likely ensue as the quantity of free dithizone approaches zero. There is also the tendency for the analyst to extract with the smallest possible excess of dithizone in order to simplify the stripping operation. This may result in incomplete extraction of lead if the pH of the solution is too low (Table 2 and Fig. 2). Therefore analysts using "one-color" methods for lead find themselves in a dilemma. If they do not remove all the unbound dithizone, their results are high, and if they take meticulous care to remove it, they lose lead in the process. Accuracy of these methods depends upon making the same compensation of errors in the preparation of standards. The greatest accuracy of "one-color" methods is about 0.001 mg., probably because these compensations cannot always be balanced. This represents a large experimental error when quantities of lead of the order of 5-20 gamma are to be determined.

The writers realize that considerable temerity is required to criticize methods accurate to 0.001 mg., but they contend that greater accuracy in the determination of small quantities of lead is attained by another application of the colorimetric dithizone principle.

#### "MIXED-COLOR" METHODS

In these methods the lead is also extracted with an excess of dithizone in chloroform solution, but the excess is allowed to partition between the aqueous and solvent fractions and so modify the color of the extract according to the relative amounts of lead and dithizone. According to this proportion a series of colors from red to green may be arranged with intermediate crimsons, purples, and blues—hence the term "mixed-color."<sup>1</sup> Carbon tetrachloride is unsuitable for "mixed-color" work as it allows the excess dithizone to partition too readily into the aqueous fraction.

<sup>1</sup> The writers realize that the term "mixed-color" has no technical significance. It is intended to indicate the color produced by the blending of the red and green components in the chloroform phase of the reaction mixture.

If the extraction is made under definite conditions of volume and strength of dithizone solution and volume and  $pH$  of aqueous fraction, the "mixed-color" obtained is definite and reproducible, and, provided excess dithizone is present, depends only upon the amount of lead.

The absorption spectra of dithizone solutions in chloroform for various degrees of saturation with lead are shown in Plate II. Visual colors range from green to red. The saturated color is seen to be a red modified by some transmission in the blue, and the absorption band at  $610\text{--}620m\mu$  is seen to disappear with increasing saturation (see also Fig. 1). The "mixed-color" method avoids the two complications introduced into the one-color methods by the extraction of excess dithizone, namely, incomplete extraction of excess dithizone and loss of lead in aqueous solutions of high  $pH$ . This is done by leaving the excess dithizone in the chloroform and by choosing the  $pH$  of operation so that there is no solubility of lead. On the basis of the previously described stability experiments, the writers believe this optimum  $pH$  to be  $9.5\text{--}10.0$ . In this range dissociation of the complex is slight and is entirely repressed by a slight excess of dithizone. Therefore the "mixed-color" method is designed to have an excess of dithizone always present, although in the upper range of any color series it may be small. The first step in attaining this optimum  $pH$  is to bring the lead into a solution of definite volume and acid strength. This solution can then be made ammoniacal with a definite volume of an ammoniacyanide solution of the proper strength to bring the resultant  $pH$  within the optimum limits for the color development as follows:

At some stage in any lead determination a point is reached where the lead can be isolated by a dithizone extraction. In most cases the lead can be extracted directly with dithizone in chloroform from a solution of the ashed or digested sample, those substances like phosphates or hydroxides which would ordinarily precipitate from ammoniacal solution being held in solution with citric acid. Or, if lead is isolated by a sulfide precipitation, the extraction can be made by redissolving the filtered sulfide, making ammoniacal, and extracting as before. In either case the color of the extracts furnishes an idea of the amount of lead to be expected. The volume of dithizone solution used is immaterial, and extraction is continued until the analyst is certain that all lead has been removed.

The lead is then brought into aqueous solution by shaking the combined chloroform extracts with 50 cc. of 1 per cent nitric acid. The chloroform solution of the now lead-free dithizone is then discarded, and the lead is left, as desired, in a definite volume of acid of definite strength. Now 10 cc. of a mixture of 75 cc. of strong ammonia and 10 grams of potassium cyanide made to 500 cc. are added, and the mixture is shaken. The resultant  $pH$  is about 9.7, and as the solution is well buffered by the cyanide, slight variations in acid strength have no effect.



This general procedure is followed unless it is desired to take aliquots of the 50 cc. of 1 per cent acid solution. If less than 50 cc. is taken, the difference is made up with pure 1 per cent nitric acid before the solution is made ammoniacal for the color development.

Standards are prepared under identical conditions of volume and pH. The standard lead solution<sup>1</sup> is made up in 1 per cent nitric acid, and volumes of standard lead solution and pure 1 per cent nitric acid are so adjusted that in all cases the total volume of acid is 50 cc. For example, in covering the range 0-10 gamma of lead it is convenient to use a solution of pure lead nitrate in 1 per cent nitric acid, 1 cc. of which contains 1 gamma of lead. The 1 gamma standard is prepared by measuring 1 cc. of standard lead solution and 49 cc. of pure 1 per cent nitric acid into a separatory funnel; the 5 gamma standard, by taking 5 cc. and 45 cc. of the respective solutions; and the 10 gamma standard by taking, respectively, 10 and 40 cc. The acid mixture is then made ammoniacal as before with the 10 cc. of ammonia cyanide reagent.

The color is developed by shaking out with a measured portion of dithizone in chloroform, whose volume and strength depend upon the lead range it is desired to cover. The same general hue progression from green to red will be observed in each lead range. Suggested volumes and concentrations of standard dithizone solutions for each range are given in Table 6.

TABLE 6.—*Volume and concentration of dithizone solution for various lead ranges*

| LEAD RANGES  | VOLUME     | CONCENTRATION |
|--------------|------------|---------------|
| <i>gamma</i> | <i>cc.</i> | <i>mg./l.</i> |
| 0- 5         | 5          | 4             |
| 0- 10        | 10         | 4             |
| 0- 20        | 10         | 8             |
| 0- 50        | 25         | 8             |
| 0-100        | 30         | 10            |
| 0-200        | 30         | 20            |

1. *Simple Color Matching.*—The simplest form of lead estimation by the "mixed-color" procedure is a visual color comparison, in tubes or vials, against standards (conveniently ten, which allows for some interpolation). It was first proposed by Vorhes and Clifford<sup>2</sup> for the determination of lead as spray residue on apples and pears and was designed for rapid routine work. Color matching against standards was done by viewing transversely in Nessler tubes. In Plate III is shown a comparator box designed to hold a complete set of standards at one time. Visually, there are two sensitive regions in the range: (1) At the lower end, where

<sup>1</sup> *This Journal*, 17, 120 (1934).

<sup>2</sup> *Ibid.*, 130.

the proportion of lead to dithizone, and hence the proportion of red to green, is small, and (2) at the upper end where the proportions are reversed.

With the general procedure outlined the colors are matched by drawing off the chloroform layer into flat-bottomed tubes or vials of appropriate size. For the low ranges the tubes are designed to give the greatest possible depth and in order to concentrate the color as much as possible the dithizone necessary to cover the range is contained in the smallest practicable volume of chloroform. Diminishing the volume and concentration of the dithizone solution restricts the range with gain in accuracy at the expense of flexibility. So marked are the color differences of a particular range that it has been possible to follow visually the ten steps of the range 0-1 gamma when the mixed colors, developed in a volume of 5 cc. of chloroform, were viewed longitudinally in narrow, flat-bottomed tubes about 6 inches long.

It should be remembered, however, that even neutral dithizone solutions appear red when the concentration or depth of column is increased beyond a certain point. Consequently, matching in the higher ranges must be done by using a more dilute dithizone solution or by viewing the colors through a shorter length; for example, transversely in tubes of matched diameter.

The simple color-matching operation is entirely practicable for quantities of lead up to 0.2 mg.

2. "*Mixed-Color*" Methods with a *Hydrogen-Ion Colorimeter*.—The great objection to simple color-matching in tubes or vials is the labor involved in making the ten standards. By using a hydrogen-ion colorimeter, the number of necessary standards is reduced to the green standard obtained with zero lead and the red standard of the top of the range. A mechanical blending of colors is secured by adjusting the vertical position of the double plunger stage.

The instrument used was the Klett-Beaver type,<sup>1</sup> and several modifications were necessary before it could be adapted to "mixed-color" work. It was necessary to use plungers longer than standard in order to keep the metal adapters from dipping into the chloroform solutions. To overcome a variation in field brightness with vertical setting of the right-hand stage, even with the cups filled with clear chloroform, it was necessary to fit a plunger of smaller diameter in the upper position, and even then it was found impracticable to work with a total depth greater than 25 mm. Finally it was not found possible to make direct hue matches, and better results were usually obtained with a monochromatic red filter in the eye-piece.

In practice the two standards are shaken out in separatory funnels and filtered into the proper cups. The light is then balanced by filtering

<sup>1</sup> *J. Optical Society Am. and Rev. Sci. Instruments*, 18, 41 (1929).

into the left-hand cup a standard developed with an amount of lead equal to one-half the range covered, and with the right-hand stage set exactly at the intermediate point the two fields are adjusted to equal intensity with the tilting mirror device. Depths are so chosen that scale readings in mm. represent some simple multiple or fraction of gamma of lead. Thus if the range 0-10 gamma is being covered with a total depth of 20 mm., a reading of 16 mm. represents 8 gamma of lead. A typical curve is given in Fig. 5.

This colorimeter was applied to the determination of lead in spray residue by the Vorhes-Clifford<sup>1</sup> method. If the total depth is set at 27 mm., readings in mm. give grains per pound direct. Some results by this technic are given in Table 7, the lead being added to the "strip solutions" of lead-free apples. Values above 0.027 gr./lb. required smaller aliquots than the prescribed 20 cc.

TABLE 7.—*Determination of lead by the Vorhes-Clifford technic with the H-ion colorimeter*

|                  |      |      |      |      |      |      |      |      |      |      |
|------------------|------|------|------|------|------|------|------|------|------|------|
| Pb added—gr./lb. | 0.37 | 0.41 | 0.26 | 0.30 | 0.08 | 0.22 | 0.15 | 0.12 | 0.18 | 0.07 |
| Pb found—gr./lb. | 0.37 | 0.40 | 0.28 | 0.30 | 0.09 | 0.21 | 0.15 | 0.14 | 0.18 | 0.08 |

Evaporation of chloroform from the cups is a decided disadvantage with colorimeter operation, and standards must be replaced frequently. With proper adjustment the hydrogen-ion colorimeter should give results accurate to 5 per cent up to a range of 0.1 mg. of lead. This is probably no more accurate than color matching in tubes, but less labor is involved.

3. "*Mixed-Color*" *Methods with a Photometer.*—The transmission spectra of the two components in the "mixed-color" mixture show a decided spread in transmission for light of wave length  $510m\mu$  (Fig. 1). Thus, if the amount of light of this wave length transmitted by the mixed components of any given lead range is measured with a spectrophotometer, absorption coefficient (used here to indicate  $-\log$  transmittancy) would be expected to increase in a linear manner with amount of lead, provided Beer's law is obeyed by both components. A photometer equipped with a blue-green filter isolating approximately this spectral band could likewise be used. Theoretically a better spread could be obtained by working at  $610m\mu$ , but the mechanics of the reaction require a small excess of dithizone to be present even at the upper end of the range to hold the lead as  $PbD_2$  in the chloroform phase. Under these conditions the so-called "saturated" color takes the form shown by the dotted line, the transmission being greatly repressed by this excess. Transmission in the green is but little affected.

For the work described in this paper the writers used a photometer and filter centered at about  $520 m\mu$ .<sup>2</sup> The light absorption-concentration curves

<sup>1</sup> *Loc. cit.*

<sup>2</sup> Later a filter centering at  $505 m\mu$  and giving a greater spread with proportionate increase in accuracy was obtained.

with this filter for the two components of the "mixed-color" are presented in Figs. 3 and 4. Thus, when the absorption coefficients of the standard colors of any given lead range, read through an appropriate cell length, are plotted against lead a nearly linear relation was observed. In practice, a batch of dithizone designed to cover a special lead range is standardized by developing the color at several points in the range with known amounts of lead. The general procedure already outlined for the preparation of standards, and the appropriate volume and concentration of dithizone (Table 6) are used. To eliminate differences in volume of extract between standards and unknowns, both the acid and the standard lead solutions used in the preparation of standards are saturated with

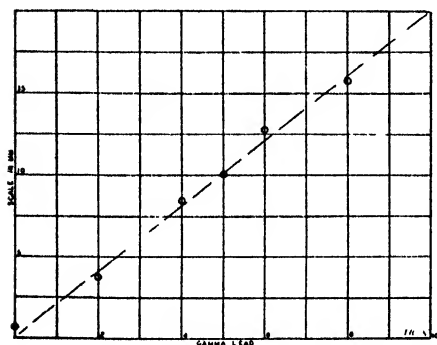


FIG. 5

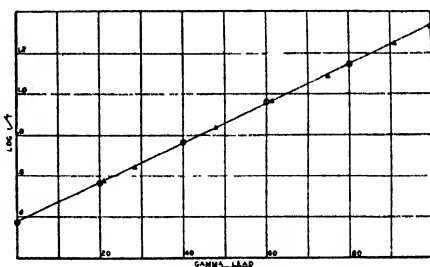


FIG. 6

FIG. 5.—DETERMINATION OF 0-10 GAMMA OF LEAD WITH THE HYDROGEN-ION COLORIMETER (DOTTED LINE REPRESENTS THEORETICAL READING).

FIG. 6.—DETERMINATION OF 0-100 GAMMA OF LEAD WITH THE PHOTOMETER AND  $\frac{1}{2}$  INCH CELL.

clear chloroform before use. The standards are filtered, and the absorption coefficient is read photometrically in the same cell. A standardization curve is thus obtained for the particular batch of dithizone. Better practice is to calculate the slope of the theoretical line connecting these standard points and the intercept for zero lead on the absorption coefficient axis. Then, the procedure for determining directly the lead content of an unknown falling within this range is to obtain the value of the absorption coefficient, subtract the intercept, and multiply by the standard factor. If protected from evaporation and direct sunlight, the standard factor of dithizone solutions should not change for at least a month.

The cells used were a 2 inch, a 1 inch, and a  $\frac{1}{2}$  inch of Pyrex glass, with plane parallel fused ends. Suggested cell lengths for the different lead ranges, with the volume and concentration of dithizone solution given above, follow:

| LEAD RANGE   | CELL LENGTH   |
|--------------|---------------|
| <i>gamma</i> | <i>inches</i> |
| 0- 5         | 2             |
| 0- 10        | 2             |
| 0- 20        | 1             |
| 0- 50        | 1             |
| 0-100        | $\frac{1}{2}$ |
| 0-200        | $\frac{1}{2}$ |

Typical standardization curves are given in Figs. 6, 7, and 8. The circles indicate the standardization points from which the theoretical line was drawn, and the triangles represent the recoveries of unknown amounts of lead. These recoveries are given below in tabular form and illustrate the accuracy obtainable by the "mixed-color" photometric method. Unknown amounts of lead were first extracted from pure solution with excess dithizone, the lead was stripped from the combined extracts with 50 cc. of the 1 per cent nitric acid, and the color was developed by making ammoniacal with the 10 cc. of standard ammonia-cyanide mixture and shaking out with the appropriate volume of standardized dithizone.

TABLE 8.—*Recoveries of lead by the "mixed-color" photometric method*

| RANGE | Pb ADDED     | Pb RECOVERED | DIFFERENCE   |
|-------|--------------|--------------|--------------|
|       | <i>gamma</i> | <i>gamma</i> | <i>gamma</i> |
| 0-100 | 21.5         | 21.2         | - .3         |
|       | 28.6         | 27.3         | -1.3         |
|       | 48.0         | 47.3         | - .7         |
|       | 61.5         | 61.2         | - .3         |
|       | 75.0         | 73.5         | -1.5         |
|       | 91.2         | 90.4         | - .8         |
|       | 110.         | 108.1        | -1.9         |
| 0- 10 | 3.48         | 3.58         | + .10        |
|       | 1.25         | 1.28         | + .03        |
|       | 5.10         | 5.00         | - .10        |
|       | 7.28         | 7.26         | - .02        |
|       | 9.21         | 9.19         | - .02        |
| 0- 1  | .1           | .08          | - .02        |
|       | .3           | .32          | + .02        |
|       | .6           | .62          | + .02        |
|       | .8           | .76          | - .04        |
|       | 1.0          | .95          | - .05        |

The necessity of extracting with an excess of dithizone is shown in Fig. 9, which contains the absorption coefficient curves for the 0-10

gamma lead range produced by 10 cc. of dithizone solution whose concentration is indicated (mg./l.) on the respective curve. Ten cc. of dithizone of 2.5 mg. per liter concentration represents about the amount equivalent to combine with 10 gamma of lead, but it is noted that considerable excess

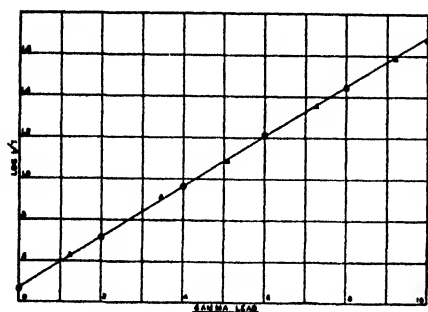


FIG. 7

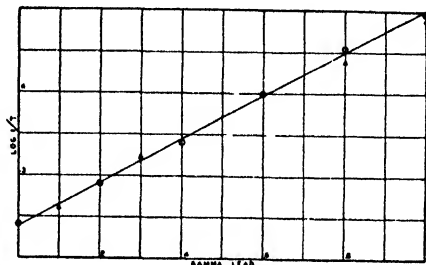


FIG. 8

FIG. 7.—DETERMINATION OF 0-10 GAMMA OF LEAD WITH THE PHOTOMETER AND 2 INCH CELL.

FIG. 8.—DETERMINATION OF 0-1 GAMMA OF LEAD WITH THE PHOTOMETER AND 2 INCH CELL.

is necessary under the conditions of the determination to hold the lead in the chloroform phase and make the curve linear. The slopes of the curves are seen to increase slightly as more concentrated dithizone solutions are used. With the volumes and concentrations of dithizone solu-

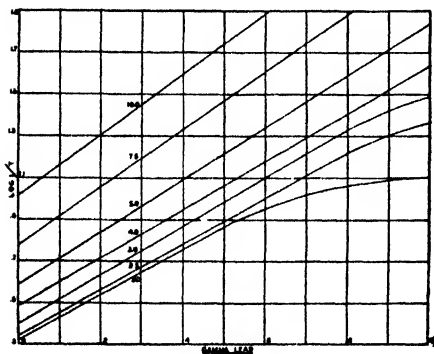


FIG. 9

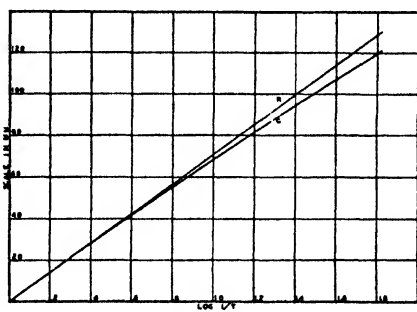


FIG. 10

FIG. 9.—CURVES ILLUSTRATING THE NECESSITY FOR EXTRACTING WITH EXCESS DITHIZONE.

FIG. 10.—CURVE "G"—CALIBRATION CURVE OF WEDGE PHOTOMETER WITH MONOCHROMATIC GREEN FILTER.

CURVE "R"—CALIBRATION CURVE OF WEDGE PHOTOMETER WITH MONOCHROMATIC RED FILTER.

tion specified in Table 6, it is possible by means of the photometer to determine quantities of lead 10 or 15 per cent greater than the top standard of the range by extrapolating the absorption curve.

The photometer used in this work is illustrated in Plate IV. It consists of a comparator head which brings the light from two equally illuminated fields together within an eye-piece. A neutral wedge-filter of light density 0.2 and 15 cm. long is mounted upon a rack and pinion in such fashion that it may be passed back and forth across one beam and thus transmit more or less light as required. A scale with vernier is fixed to the rack so that the position of the wedge may be closely noted. The wedge itself, after being mounted in position, is calibrated by means of the monochromatic green filter, with a series of neutral filters of accurately known transmission. Before calibration the wedge is adjusted to read zero with a cell full of clear solvent in place, and this cell is kept in place during the calibration of the wedge. Thus losses by reflection from the cell windows are compensated for, and the 0 of the scale corresponds to unit transmittancy or 0 absorption coefficient. A large scale graph is then made to give scale reading in terms of absorption coefficient ( $-\log T$ ). The calibration curve for the wedge used in this work is given in Fig. 10. With the green filter, the scale-absorption coefficient relationship is not linear, but with a red filter it is almost exactly so. These wedges are not entirely permanent; however, there has been no perceptible change in calibration during seven months of constant use, and with occasional recalibration they should be serviceable for a period of several years. The light source is a magnesium oxide coated plate strongly illuminated by six 100 watt lamps. This gives an even diffused illumination. To protect the wedge from excessive heat a current of air may be drawn through the wedge box during use, and its temperature occasionally noted by means of the thermometer.

In practice the unknown solution is placed in the trough and the wedge adjusted until a balance is obtained. Very precise settings can be made. Scale reading is noted, and the corresponding absorption coefficient is read from the chart.

A calibration of the wedge is not entirely necessary, and a standard curve plotting simple scale reading against amount of lead may be constructed for each batch of dithizone. (Scale readings with the green filter are nearly linear with absorption coefficient.)

In adapting the neutral wedge photometer to the determination of lead on apples by the Vorhes-Clifford method the original technic was followed except that amounts of lead in the standards were halved and 10 cc. aliquots of strip solution (instead of 20) were used in the determination. Standards were prepared by mixing "blank" and "standard" solutions as follows:

|                         |    |      |      |      |      |
|-------------------------|----|------|------|------|------|
| Lead (gr./lb.)          | .0 | .006 | .012 | .018 | .024 |
| Standard solution (cc.) | 0  | 2.2  | 4.4  | 6.7  | 8.9  |
| Blank solution (cc.)    | 20 | 17.8 | 15.6 | 13.3 | 11.1 |

These proportions of blank and standard were measured into separatory funnels, and the color was developed by shaking out with 20 cc. of dithizone of 15 mg. per liter concentration. The chloroform layers were filtered and read in a  $\frac{1}{2}$ -inch cell, and the curve (Fig. 11), plotting scale reading against gr./lb., constructed.

Recoveries were checked by adding known amounts of lead to the strip solution of lead-free apples, taking appropriate sized aliquots (making up to 20 in all cases with blank solution), making alkaline with the standard

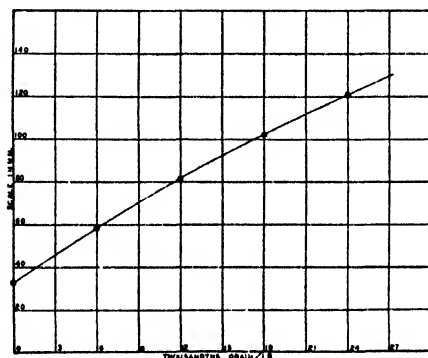


FIG. 11.—USE OF PHOTOMETER IN DETERMINATION OF LEAD IN SPRAY RESIDUE BY THE VORHES-CLIFFORD METHOD.

ammonia mixture, and shaking out in a separatory funnel with the standard dithizone. Scale reading with the  $\frac{1}{2}$  inch cell was determined, and the corresponding amount of lead was obtained from the curve. The results are given in Table 8.

TABLE 8.—*Determination of lead by the Vorhes-Clifford technic with the photometer*

|                    |     |     |     |     |     |     |     |     |     |     |
|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pb added (gr./lb.) | 005 | 010 | 015 | 020 | 025 | 030 | 035 | 040 | 045 | 050 |
| Pb found (gr./lb.) | 005 | 010 | 015 | 020 | 025 | 031 | 035 | 040 | 046 | 051 |

The advantage of the photometric method in the determination of spray residue lead by this procedure is that the labor involved in the daily preparation of standards is eliminated, and the standardization curve may be used as long as the batch of dithizone lasts. Furthermore, ability of the operator to distinguish slight color differences is not involved as in the direct color-matching method. The only requirement is an easily made brightness match in a monochromatic field.



The "mixed-color" method with photometric estimation of lead has been applied to a variety of materials. The writers prefer the ashing method of sample preparation<sup>1</sup> and use either the dithizone extraction or sulfide precipitation for preliminary isolation of the lead. To illustrate, it is proposed to outline a procedure for the determination of lead in urine for which the dithizone extraction was used, and also one for this determination in raw bone, for which the sulfide separation is preferable.

#### APPARATUS AND MATERIALS

Redistil all water and use porcelain vessels and Pyrex glassware. Clean new glassware with warm 10% NaOH solution, then with hot HNO<sub>3</sub>, and finally rinse thoroughly with redistilled water. Clean separatory funnels by shaking out immediately before use with a mixture of dilute ammonia and dithizone solution, then with dilute HNO<sub>3</sub>, and finally rinse thoroughly.<sup>2</sup> Remove traces of lead and acid from filter papers through which the chloroform solutions are filtered before photometric measurement by soaking overnight in 1% HNO<sub>3</sub>, washing with large volumes of redistilled water on a Büchner funnel, and drying. (This precaution is necessary as the color may be discharged or altered by the traces of acid and lead usually present in even the best grade of paper filters.) For other filtrations use fritted glass filters of appropriate size, cleaning with hot HNO<sub>3</sub> before use.

#### REAGENTS

Store all reagents in Pyrex containers.

(a) *Hydrochloric acid*.—Redistil the concentrated reagent to obtain the constant boiling mixture.

(b) *Nitric acid*.—Redistil the concentrated reagent, boil off nitrous fumes, and readjust to 1.40 sp. gr. by either evaporation or dilution.

(c) 1 per cent *nitric acid*.—Make 10 cc. of reagent (b) to 1 liter with redistilled water.

(d) *Citric acid*.—Bring a strong solution of the C.P. reagent to pH 3.5–4.0 with ammonia, add a little copper salt, and pass in H<sub>2</sub>S. Let stand and filter. Boil off every trace of H<sub>2</sub>S and adjust to convenient concentration.

(e) *Ammonia*.—Redistil the concentrated reagent, passing the evolved gas into ice-cold redistilled water. Determine the sp. gr. of the distillate.

(f) *Potassium cyanide solution*.—10 per cent W/V.

(g) *Ammonia-cyanide mixture*.—Make to 500 cc. a volume of solution (e) equivalent to 75 cc. of strong (28.3%; .90 sp. gr. at 15° C.) ammonia plus 100 cc. of solution (f).

(h) *Ash-aid solution*.—Prepare lead-free Ca(NO<sub>3</sub>)<sub>2</sub> · 5H<sub>2</sub>O by sulfiding a strong solution of the salt, filtering, boiling off H<sub>2</sub>S, and recrystallizing. Use the same procedure with Al(NO<sub>3</sub>)<sub>3</sub> · 9H<sub>2</sub>O, bringing the pH of a strong solution up to 3.0–3.2 before sulfiding with ammonia. (The precipitate formed by local action of the ammonia will redissolve, at this pH, upon shaking.) Dissolve 20 grams of the calcium salt plus 40 grams of the aluminum salt in 100 cc. of redistilled water.

(i) *Dithizone in chloroform*.—For extraction, add 10–20 mg. to 1 liter of redistilled chloroform.

(j) *Standard dithizone solutions*.—Concentrations have been given for the various ranges. It will be found convenient to have on hand standardised dithizone solutions for the ranges 0–5, 0–20, and 0–100 gamma of lead.

<sup>1</sup> *This Journal*, 18, 815 (1935).

<sup>2</sup> Wilkens, Willoughby, et al. (*Loc. cit.*).

(k) *Indicators*.—0.04 per cent bromphenol blue and 0.04 per cent thymol blue.

(l) *Copper solution*.—Dissolve 1 gram of electrolytic copper in minimum  $\text{HNO}_3$  and make to 500 cc.

#### PROCEDURE

*Urine*.—Measure 100 cc. of urine into a 9 cm. casserole and bring to dryness on the steam bath. Put into a temperature-controlled muffle at  $500^\circ \text{C}$ . and char for about 15 minutes. Remove, and add 2–3 cc. of the ash-aid solution dropwise, so as to moisten all portions of the char. (If concentrated  $\text{HNO}_3$  is used as ash-aid at this stage, deflagration will result.) Dry past danger of spattering and replace in the muffle. If the ash is not clean within 30 minutes, remove, cool, and regenerate the nitrates with 2 cc. of  $\text{HNO}_3$  (b). Dry, and replace in the muffle, ashing until clean.

Remove the casserole, cool, add 10 cc. of the  $\text{HCl}$  and bring to dryness. Add another 10 cc. portion and again bring nearly to dryness. (This treatment with  $\text{HCl}$  is necessary in order to bring refractory ash salts completely into solution.) Take up with water and rinse into a separatory funnel with enough water to make the total volume used 100 cc. Add a quantity of the citric acid solution equivalent to 5 grams of citric acid and bring to about pH 8.5 with a measured volume of the ammonia (e). Cool, add 5 cc. of the  $\text{KCN}$  solution, and extract with two 20 cc. portions of the dithizone in  $\text{CHCl}_3$ , adding the first portion-wise, with shaking between additions to obtain a rough idea of the quantity of lead present. (These amounts are usually sufficient for complete extraction of the lead. Additional portions may be necessary when large amounts are encountered.) Combine the extracts and strip with two consecutive 25 cc. portions of the 1%  $\text{HNO}_3$ , using the second portion to wash out the funnel in which the first stripping was made. Filter the acid extracts in succession through a small pledget of moist acid-washed cotton inserted in the stem of a small funnel into a 50 cc. graduated flask or cylinder. (This procedure removes small globules of dithizone solution which persist in the aqueous fraction and change the volume and strength of the  $\text{CHCl}_3$  extract in the final color development. This error is inconsequential when the color development is made with large volumes of standard dithizone, as when working in the higher lead ranges (0–100 gamma). Here the acid extract is washed with two small portions of clear  $\text{CHCl}_3$ , drained off as cleanly as possible, and the color is developed in the same separatory funnel. If desired, the lead may be stripped from the chloroform extracts with one 50 cc. portion of the 1%  $\text{HNO}_3$  solution. This eliminates the use of an extra separatory funnel. The acid solution is filtered, as before, into a 50 cc. cylinder, and any slight deficiency (usually 0.5–1 cc.) corrected for in the final result.) Make up any slight deficiency with a few drops of the 1%  $\text{HNO}_3$ . Transfer the entire 50 cc. (or aliquots, the difference being made to 50 with the same solution) to another separatory. Add 10 cc. of solution (g) and shake the mixture. Add the appropriate volume of standardized dithizone, depending upon the range in which it is desired to work, and shake the mixture for 1 minute. If the range is exceeded, as evidenced by the color of the chloroform fraction, re-extract with an excess of the dithizone in  $\text{CHCl}_3$ , strip as before with 50 cc. of the 1%  $\text{HNO}_3$ , and develop the color as before with standardized dithizone covering a higher range. Allow the funnel to stand for a few minutes after the color development, filter off the chloroform layer through the specially prepared filters, and fill into the proper cell. Folding a 9 cm. filter directly into the mouth of a 50 cc. low-form beaker eliminates the necessity for a funnel in the filtration. Determine the absorption coefficient and calculate the amount of lead present from the standard factor of the dithizone.

*Bone*.—Char a 5 gram sample for 1 hour at  $500^\circ \text{C}$ . Remove the casserole, cool, and wet with 2 cc. of  $\text{HNO}_3$  (b), breaking up lumps with a stirring rod. Dry and

replace in the muffle until clean. Cool, add 10 cc. of the HCl, and evaporate to dryness. Take up with another 10 cc. portion of the HCl and transfer to a glass-stoppered 200 cc. Erlenmeyer flask with 100 cc. of hot water. Add a sufficient quantity of the citric acid solution to equal 10 grams of citric acid and bring to pH 3.4–3.5 with a measured volume of the ammonia, cooling and shaking between additions in order to redissolve precipitated phosphates. Add 1 cc. of the Cu solution and pass in  $H_2S$  for 5 minutes, filtering the gas through a plug of tightly packed cotton in an absorption tube. Filter the solution immediately (the sulfides will coagulate readily at this pH and may be filtered immediately) through a fritted glass filter, discarding the filtrate. Do not wash the precipitate. Take up with 5 cc. of warm  $HNO_3$  (b), wetting all portions of the filter, allow to stand a few minutes, and draw through into the original flask, washing the filter with 50 cc. of hot water added portion-wise. Stopper the flask, shake, and then boil for a few minutes to remove traces of  $H_2S$ . Transfer to a separatory funnel, add citric acid solution equivalent to 5 grams of citric acid, and adjust the pH to about 8.5 with a measured amount of the ammonia. Add 5 cc. of the KCN solution and proceed as directed under urine.

#### BLANKS ON REAGENTS

The lead blank of the determination becomes increasingly important when the smaller amounts of lead are being determined. The writers have been unable to reduce this blank below 0.001 mg., in spite of all reasonable precaution. Part of the difficulty is in purification of reagents, but there is also the problem of storage. Pyrex is not ideal for this purpose as it contains traces of lead. It is significant that it was impossible to obtain lead-free water from an all-Pyrex still, and after repeated redistillation the lead content ranged from 2 to 4 gamma per liter. The amount increased gradually upon storage. Contamination with lead-bearing dust is another factor. Hence it is necessary to make most careful blank determinations upon the exact amount of reagents (even water) used in the determination and to expose the blank and the sample for the same length of time on the steam bath or in the muffle. When lead determinations are made upon aliquots of the 50 cc. of 1 per cent nitric acid in which the lead is isolated preparatory to the color development, only a corresponding part of the total blank should be subtracted. Accuracy of results thus depends upon the accuracy of two determinations, lead in the sample and lead in the blank. It is doubtless possible to further reduce the blank, but the cost of silica stills and storage vessels may be prohibitive. Figures on a variety of material showing lead recoveries when known amounts were carried through one or the other of the above procedures are given in Table 9.

#### DISCUSSION

The results reported here warrant the conclusion that the "mixed-color" dithizone method can be applied to the determination of lead in any food or biological material. A combination of this principle with photometric methods of measurement has resulted in more satisfactory results than any obtained formerly. This "mixed-color" principle should

TABLE 9.—*Recoveries of lead by "mixed-color" photometric method*  
(Amounts of lead in gamma)

| MATERIAL  | PROCEDURE FOR ISOLATION OF LEAD | REAGENT BLANK | TOTAL BLANK | LEAD FOUND | LEAD FOUND LESS TOTAL BLANK | RECOVERY        |
|---|---------------------------------|---------------|-------------|------------|-----------------------------|-----------------|
|   |                                 |               |             |            |                             | <i>per cent</i> |
| 100 cc. urine   | Extraction                      | 1.04          |             | 2.71       | 1.67                        | —               |
| +2 Pb   | "                               |               | 2.71        | 4.51       | 1.80                        | 90              |
| +4 Pb   | "                               |               |             | 6.77       | 4.06                        | 101             |
| +6 Pb   | "                               |               |             | 8.90       | 6.19                        | 103             |
| +8 Pb   | "                               |               |             | 10.73      | 8.02                        | 100             |
| 50 cc. milk <sup>1</sup>                              | Extraction                      | 1.54          |             | 2.32       | .78                         | —               |
| +1 Pb   | "                               |               | 2.32        | 3.36       | 1.04                        | 104             |
| +2 Pb   | "                               |               |             | 4.40       | 2.08                        | 104             |
| +3 Pb   | "                               |               |             | 5.21       | 2.89                        | 96              |
| 20 cc. beef blood <sup>1</sup>                        | Extraction                      | 1.39          |             | 3.64       | 2.25                        | —               |
| +4 Pb   | "                               |               | 3.64        | 7.92       | 4.28                        | 107             |
| +8 Pb   | "                               |               |             | 11.32      | 7.68                        | 96              |
| +12 Pb  | "                               |               |             | 15.50      | 11.86                       | 99              |
| +16 Pb  | "                               |               |             | 19.20      | 15.56                       | 97              |
| 10 g. Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> | Sulfide and Extraction          |               |             | 1.34       | —                           | —               |
| +4 Pb   | "                               |               | 1.34        | 5.51       | 4.17                        | 104             |
| +8 Pb   | "                               |               |             | 9.22       | 7.88                        | 97              |
| +12 Pb  | "                               |               |             | 13.42      | 12.08                       | 101             |
| +16 Pb  | "                               |               |             | 17.51      | 16.17                       | 101             |
| 10 g. cocoa   | Sulfide and Extraction          | 1.5           |             | 3.5        | 2.0                         | —               |
| +10 Pb  | "                               |               | 3.5         | 11.5       | 8.0                         | 80              |
| +30 Pb  | "                               |               |             | 31.6       | 28.1                        | 94              |
| +50 Pb  | "                               |               |             | 50.2       | 46.7                        | 93              |
| +70 Pb  | "                               |               |             | 69.3       | 65.8                        | 94              |

<sup>1</sup> Sample drawn directly into Pyrex.

also apply to the determination of small amounts of other metals. In a recent paper H. Fischer<sup>1</sup> has given absorption spectra for most of the dithizone metal complexes, and a study of these in connection with the absorption spectrum of pure dithizone will show the investigator whether or not the "mixed-color" principle will apply to any particular metal. Stability experiments, as described in this paper, will show the optimum pH of operation.

Interferences of bismuth and tin have not been discussed, but it might be well to indicate points in the procedure where methods for their

<sup>1</sup> *Wiss. Veröffentlich. Siemens-Werken*, 14, 2, 41 (1935).

elimination could be applied. The writers suggest the previously mentioned dithizone extraction at pH 2.0 for removal of bismuth preliminary to the alkaline isolation of the lead.<sup>1</sup> It may also be possible to remove tin in this manner. If not, the writers suggest a complete preliminary extraction, under alkaline conditions, with excess dithizone, stripping of the dithizone extracts with dilute hydrochloric acid and evaporation of the acid extract to dryness. The residue can then be treated at steam bath temperature with several portions of the HBr-Br<sub>2</sub> reagent of Fischer<sup>2</sup> to volatilize tin, taken up in nitric acid, and given a further preliminary extraction with excess dithizone to isolate lead.

More satisfactory results by the "mixed-color" method await the application of more precise photoelectric methods of color measurement, the use of longer cells, and especially the reduction of the reagent blank.

#### SUMMARY

(1) Some physical properties of dithizone are presented, and the mechanism of the lead dithizone reaction is discussed.

(2) Dithizone methods are classified, and certain inherent errors are indicated.

(3) The "mixed-color" dithizone method, previously applied to the determination of lead as spray residue on fruits only, is modified to make it more generally applicable.

(4) A photometric method of measuring the color, which increases the sensitivity and accuracy of dithizone methods for lead, is presented.

(5) The accuracy of the method is illustrated by results obtained on several foods and on biological materials.

#### ACKNOWLEDGMENT

The writers are indebted to Dr. Brooks A. Brice, at whose suggestion the neutral wedge photometer was constructed, for assistance in developing the photometric method of color measurement herein described.

### A MULTIPLE-UNIT DISTILLING APPARATUS FOR DETERMINATION OF FLUORINE BY THE WILLARD AND WINTER METHOD

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and Soils, Washington, D. C.)

During the distillation of fluorine with perchloric acid by the Willard and Winter method<sup>3,4</sup> it is necessary to maintain the boiling point of the solution at 120°–150° C. In the method as originally outlined, the dis-

<sup>1</sup> Willoughby, *Loc. cit.*

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Willard and Winter, *Ind. Eng. Chem. Anal. Ed.*, **5**, 7–10 (1933).

<sup>4</sup> Winter and Butler, *This Journal*, **16**, 105–7 (1933).

tilling flask is heated directly by means of a gas burner, and the boiling point of the solution is regulated by allowing water to run into the flask from a dropping funnel. Under these conditions, the regulation of the boiling point of the solution requires such close attention by the analyst that it is not advisable to run more than two distillations simultaneously, and only about ten determinations can be made per day. Furthermore, considerable bumping of the solution frequently occurs during the distillation of certain materials, particularly those that contain acid-decomposable silicates.

Dr. Simon Klosky,<sup>1</sup> of the American Agricultural Chemical Company, advised the writers, about the first of 1935, that his laboratory had overcome these difficulties to a large extent by heating the distilling flask in an oil bath and continuously adding water as steam from a regulated source of supply. The advantages of this procedure were confirmed by the writers, and it was also found that very satisfactory results were obtained by heating the distilling flask directly in a vertical electric furnace maintained at a temperature of approximately 200° C. Further experiments led to the development of an electrically heated distilling apparatus in which six distillations can be made simultaneously with comparatively little attention by the analyst. The apparatus has been in use for nearly one year, without requiring any repairs, during which time approximately 1500 distillations have been made, principally on samples of phosphate rock and calcined phosphate.<sup>2</sup>

#### DESCRIPTION OF APPARATUS

The construction of the apparatus is shown in Figs. 1 and 2, which are, for the most part, self-explanatory. Referring to Fig. 1, the top, sides, ends, and bottom of the heater, and the shelf that supports the steam generating flasks (*A*) are made of Transite board. The ends are cut from solid board and serve as supports for the heater. The top is provided with a series of removable covers, divided at the centers of the openings through which pass the necks of the distilling flasks (*B*).

In Fig. 1, the heating unit (*C*) consists of 50 feet of No. 24 B and S gage Nichrome wire (No. 5) wound in a coil supported in four longitudinal grooves on a Transite board, and covered with a thin perforated Transite board. The unit, which has a resistance of approximately 50 ohms, is designed to operate on 220-volt current and is controlled by the switch (*G*) and rheostat (*H*) shown in Fig. 2. The coil of Nichrome wire is stretched more at the center of the heating unit than at the ends in order to obtain a more even distribution of the heat.

The distilling flasks (*B*) are standard long-necked 50 ml. flasks, altered as shown in Fig. 1, and connected with individual 300 ml. flasks (*A*),

<sup>1</sup> Private communication.

<sup>2</sup> Reynolds, Jacob, and Rader, *Ind. Eng. Chem.*, 26, 406-12 (1934); Reynolds, Jacob, Marshall, and Rader, *Ibid.*, 27, 87-91 (1935); Marshall, Reynolds, Jacob, and Rader, *Ibid.*, 27, 205-9 (1935).

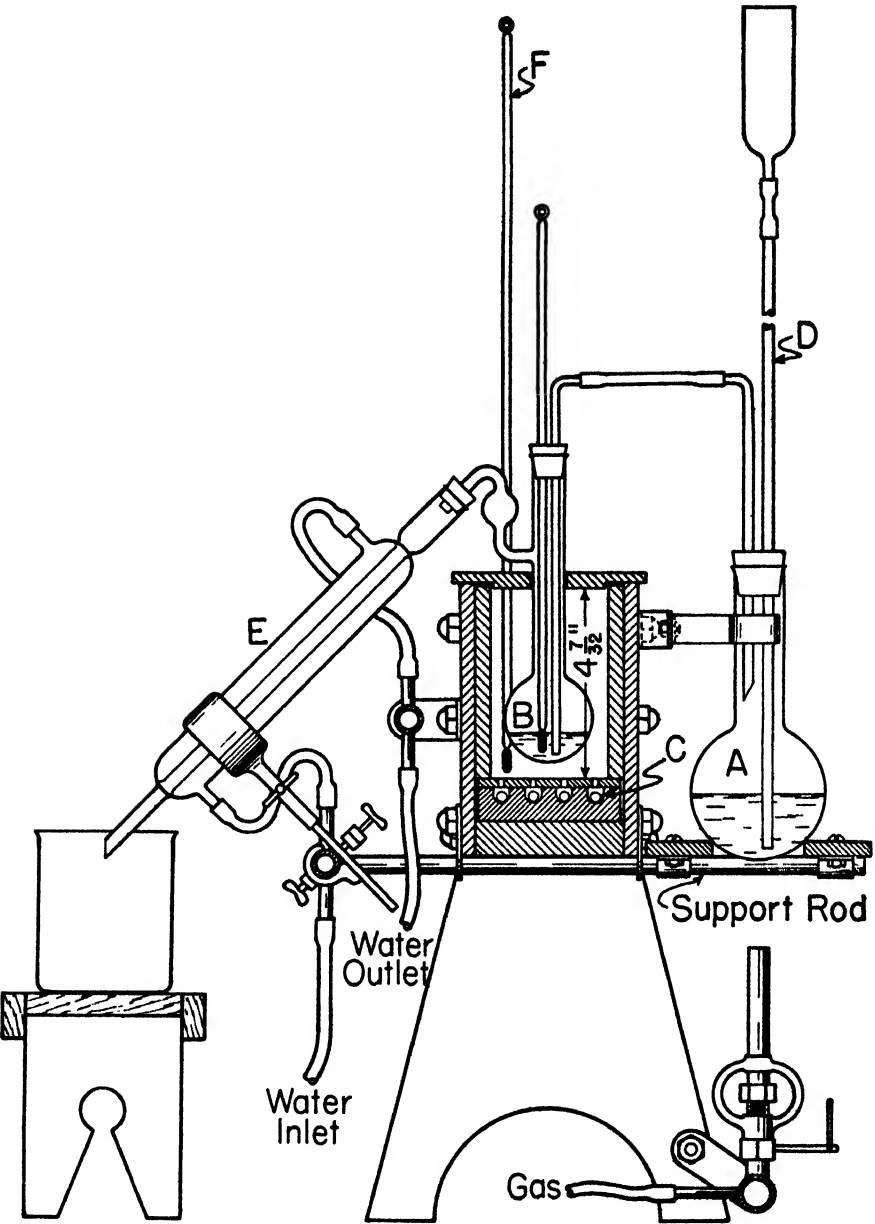


FIG. 1.—DISTILLING APPARATUS FOR DETERMINATION OF FLUORINE.

which serve as steam generators and are equipped with safety tubes (*D*). The condensers (*E*) are connected so that three are in series and the two series in parallel. The temperature of the heater is measured by means of a thermometer (*F*) placed midway between the two end walls. The temperature at this point is maintained at about 210° C.

#### DETERMINATION OF FLUORINE

With this apparatus the writers have used the following procedure with satisfactory results for the analysis of a wide variety of materials, particularly phosphate rock, calcined phosphate, and superphosphate.

As indicated in papers by Reynolds<sup>1</sup> and Shuey,<sup>2</sup> it seems desirable to use small samples for analysis. With finely ground and uniformly mixed samples of phosphate rock and calcined phosphate, containing 2 per cent or more of fluorine, a 0.1 gram sample is sufficient. If the sample contains less than 2 per cent of fluorine, satisfactory results are usually obtained with a 0.2 gram sample.

With the particular apparatus used by the writers, the temperature at the ends of the heater is somewhat lower than that at the center, which necessitates an adjustment of the conditions of distillation in order to obtain approximately uniform temperatures in all the distilling flasks. This is accomplished, to a certain extent, by using concentrated perchloric acid (10 ml. of 60 per cent acid) in the first and sixth distilling flasks and weaker acid (10 ml. of 60 per cent acid plus 5 ml. of water) in the other flasks. The details of the procedure follow:

Connect the flasks to the condensers and heat the contents to 80°–90° C. before admitting steam. Regulate the flow of steam so that 150 ml. of distillate is collected in 50–60 minutes, and maintain the temperature in the flask at 120°–150° C. during the greater part of the distillation. If the temperature in the distilling flask exceeds 150° C. during the course of the distillation, add sufficient water, through the rubber connection to the steam generator, to reduce the temperature to 120°–130° C.

Make the distillate slightly alkaline with 5% sodium hydroxide solution, using either litmus paper or one drop of phenolphthalein as an indicator; evaporate to about 5 ml., and transfer with 2 to 3 small washings to a glass cylinder approximately 10 cm. long and 2.5 cm. in diameter. Add 2 drops of a suitable indicator solution (either a mixed zirconium-alizarin indicator<sup>3</sup> or a 0.05% aqueous solution of sodium alizarin sulfonate<sup>4</sup>); discharge the red color with a slight excess (2 to 3 drops) of 0.1 *N* HCl; mix the solution with an equal volume of 95% alcohol and titrate with thorium nitrate solution (either 0.01 *N* or 0.04 *N*) to a permanent pink color. Near the end point stir the solution vigorously and allow to stand for a minute or more after each small addition of thorium nitrate.

When the amount of fluorine in the solution is more than 1.0 mg. make the titration with 0.04 *N* thorium nitrate, using the mixed zirconium-alizarin indicator. With smaller amounts of fluorine it is preferable to use the 0.05% aqueous solution

<sup>1</sup> *This Journal*, 17, 323–9 (1934); 18, 108–13 (1935).

<sup>2</sup> *Ibid.*, 17, 149–56 (1934).

<sup>3</sup> Willard and Winter, *Loc. cit.*; Reynolds, *This Journal*, 17, 323–9 (1934).

<sup>4</sup> Armstrong, W. D., *J. Am. Chem. Soc.*, 55, 1741–2 (1933).



of sodium alizarin sulfonate and titrate with 0.01 *N* thorium nitrate. The indicator correction amounts to  $-0.03$  ml. of 0.01 *N* thorium nitrate with the 0.05% solution of sodium alizarin sulfonate and to  $+0.02$  ml. of 0.04 *N* thorium nitrate with the mixed zirconium-alizarin indicator. Standardize the thorium nitrate solutions against pure sodium fluoride at approximately the same concentration of fluorine and total volume of solution as occurs in the average determination.

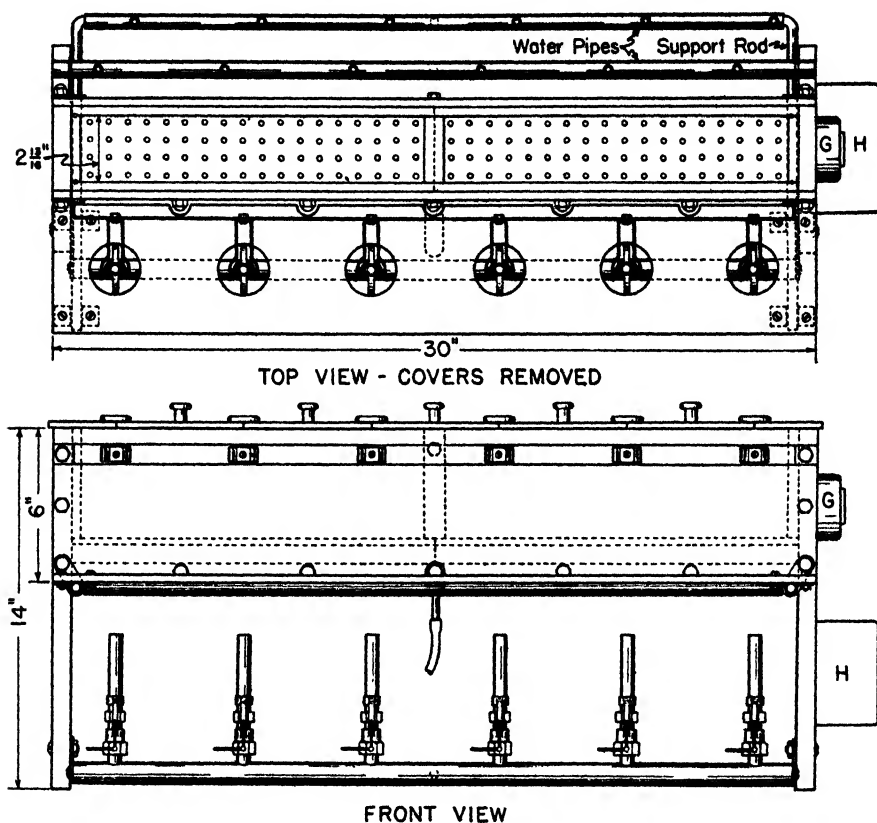


FIG. 2.—DISTILLING APPARATUS FOR DETERMINATION OF FLUORINE.  
TOP AND FRONT VIEWS.

As Reynolds<sup>1</sup> points out in a previous paper, the coating of precipitated silica which gradually accumulates on the walls of the distilling flasks should be removed at frequent intervals by means of concentrated sodium hydroxide solution.

#### PERFORMANCE OF DISTILLING APPARATUS

Results obtained with the electric distilling apparatus are shown in Table 1, in comparison with those obtained by distillation over a gas burner with introduction of the water from a dropping funnel. The pro-

<sup>1</sup> *This Journal*, 18, 108-13 (1935)

cedures differed only in the method of heating the distilling flask and introducing the water. The figures are the average of four, or more, closely-agreeing determinations. In general, the results by the two pieces of apparatus show close agreement, with a tendency toward slightly higher results by distillation over a gas burner.

TABLE 1.—*Performance of distilling apparatus*  
(Results expressed in percentage of the sample)

| MATERIAL                                    | FLUORINE                 |                          |
|---|--------------------------|--------------------------|
|   | APPARATUS A <sup>a</sup> | APPARATUS B <sup>b</sup> |
| Florida land-pebble phosphate               | 3.78                     | 3.77                     |
| Florida hard-rock phosphate                 | 3.89                     | 3.86                     |
| Florida waste-pond phosphate                | 2.19                     | 2.16                     |
| Florida waste-pond phosphate                | 2.03                     | 2.05                     |
| Tennessee brown-rock phosphate              | 3.75                     | 3.73                     |
| Tennessee brown-rock phosphate <sup>c</sup> | 3.59                     | 3.60                     |
| Tennessee blue-rock phosphate               | 3.87                     | 3.96                     |
| Wyoming phosphate                           | 3.58                     | 3.57                     |
| Electric phosphoric acid furnace slag       | 3.39                     | 3.37                     |
| Electric phosphoric acid furnace slag       | 3.43                     | 3.39                     |
| Calcined phosphate                          | 1.12                     | 1.14                     |
| Calcined phosphate                          | 1.21                     | 1.15                     |
| Calcined phosphate                          | 0.20                     | 0.18                     |

(a) Distilling flask heated over gas burner; water introduced from dropping funnel.

(b) Distilling flask heated in electric heater; water introduced as steam.

(c) Bureau of Standards sample No. 56.

Reynolds<sup>1</sup> has shown that the distillate from samples heated in the usual manner over a gas burner invariably contains small quantities of phosphoric oxide when (1) phosphoric acid is used as the distilling agent, or (2) large samples (about 3 grams or more) of phosphate are distilled with perchloric acid. Under the same conditions, the distillates obtained with the electric distilling apparatus also contain small quantities of phosphoric oxide. Furthermore, the occurrence of phosphoric oxide in the distillate is not eliminated by distilling the sample from a modified Claissen flask in which the side-arm is connected to the condenser through a vertical Kjeldahl trap. The phosphoric oxide in the distillate forms insoluble thorium phosphate with the thorium nitrate and the results for fluorine are correspondingly high. The error can be eliminated by (1) neutralizing the distillate, evaporating to a small volume, and re-distilling with perchloric acid, or (2) correcting the results for the fluorine equivalent to the phosphoric oxide in the distillate.

The distillates do not contain phosphoric oxide when small samples (0.1–0.2 gram) of phosphate are distilled with perchloric acid.

<sup>1</sup> *This Journal*, 12, 108–12 (1935).

## SUMMARY

An electrically-heated multiple-unit distilling apparatus for the determination of fluorine by the Willard-Winter method is described and illustrated. With this apparatus, 6 distillations can be made simultaneously, and 24 determinations can be completed in 8 hours. The performance of the apparatus on typical samples of phosphatic materials is indicated.

## METHODS FOR DETERMINING CARBON DIOXIDE IN BEER AND CARBONATED BEVERAGES\*

By PHILIP P. GRAY and IRWIN M. STONE  
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With few exceptions the range in the carbon dioxide content of American beers and ales is between 0.37 and 0.50 per cent, by weight, equivalent to 1.9–2.6 volumes of carbon dioxide, referred to standard conditions, per volume of beer at 25° C. and atmospheric pressure. Within this relatively narrow range there exists the possibility for considerable variation in the flavor, appearance, and "life" of the beverage at the time of consumption, depending upon other factors, such as composition, temperature of dispensing, or pouring, agitation, etc. Moreover, an important characteristic of beer, its foam volume and foam stability, is dependent to a large extent upon its carbon dioxide content. Under-carbonation yields a "flat" beer with little foam, and over-carbonation may have equally disastrous results in the shape of "wild" beer, as regards the product's appeal to the consumer. In spite of the importance of the careful control that such a situation demands, no wholly satisfactory analytical method for the accurate determination of carbon dioxide exists.

A simple control method in common use in the carbonated beverage industry consists in reading the gas pressure on a gage after piercing the crown, whereupon the volume ratio of carbon dioxide is ascertained from tables furnishing solubility data for carbon dioxide in water at different pressure and temperature levels. This method undoubtedly has the advantage of extreme simplicity, but, as shown later, it cannot be relied upon, partly for the reason that the solubility data on which such tables are based refer to the solubility of carbon dioxide in water and not in beer, but mainly because no allowance is made for the influence of the partial pressure of the air present.

## CHEMICAL METHODS

*Gravimetric methods.*—Among these may be mentioned the method of Macheleidt<sup>1</sup> and a modification suggested by Bode and Hembd.<sup>2</sup> The

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, November, 1935.

<sup>1</sup> *Z. ges. Brauw.*, 130 (1921); *J. Inst. Brew.*, 522 (1921).

<sup>2</sup> *Wochschr. Brau.*, 51, 60 (1934).

beer is made alkaline with ammonia. The phosphates are precipitated with magnesia mixture and filtered off. The carbonates are then precipitated as calcium carbonate, filtered, washed, and ignited. The carbon dioxide content is calculated from the weight of calcium oxide. This method is obviously lengthy and unwieldy. Bode<sup>1</sup> also, much earlier, suggested another gravimetric method, which consisted in equipping the beer bottle with drying tubes, adding pumice and lard, and then determining the carbon dioxide by the loss in weight of the sample after boiling. Bode's method appears to be the only one that involves the addition of some material to the beer to control the evolution of the gas and the use of a defoaming agent.

*Gasometric methods.*—Reference is made to the methods of Wiebold,<sup>2</sup> Baker and Hulton,<sup>3</sup> and Lundin, Ohlin, and Ellburg.<sup>4</sup> Here the carbon dioxide is liberated from the sample by gradual heating, and the gas is collected for measurement. In the last-named method the Van Slyke apparatus is used, a charge of beer being taken by means of a special crown-piercing sampler. The disadvantages of such gasometric procedures are the specialized technic necessary to obtain accurate results and the errors introduced by small samples for a material like beer.

*Chemical volumetric methods.*—Gawalowski<sup>5</sup> passes the gas from a bottle of beer into barium hydroxide and subsequently titrates the carbon dioxide absorbed by the barium hydroxide. Van Laer<sup>6</sup> passes the carbon dioxide into ammonia and then precipitates calcium carbonate from this ammoniacal solution. The calcium carbonate is filtered off and titrated with standard sulfuric acid. These methods involve considerable trouble, due to foaming, quite aside from the inaccuracies introduced through incomplete recovery of carbon dioxide and inconvenient manipulations.

Blom and Hand<sup>7</sup> modified the original method of Blom and Krause,<sup>8</sup> in which sodium hydroxide is added to the beer to fix carbon dioxide, a sample is then removed and acidified, and a current of carbon dioxide-free air is bubbled through the liquid, the aspirated carbon dioxide being absorbed by standard barium hydroxide solution and then subjected to titration. Cannizzaro<sup>9</sup> describes the simple method of pipetting a sample of cold beer into standard sodium carbonate and titrating this solution to the phenolphthalein end point in order to determine the amount of bicarbonate formed from the carbon dioxide. DeClerck<sup>10</sup> modifies this method by substituting sodium hydroxide for the sodium carbonate and using two indicators. In this simple method the end points tend to be

<sup>1</sup> *Wochschr. Brau.*, 21, 510 (1904).

<sup>2</sup> *Chem. Z.*, 101 (1906).

<sup>3</sup> *J. Inst. Brewing*, 40, 171 (1934).

<sup>4</sup> *Wochschr. Brau.*, 45, 339 (1928); 47, 121, 137 (1930).

<sup>5</sup> *Z. Kohlenäureind.*, 4, 409 (1898); *J. Inst. Brewing*, 202 (1899).

<sup>6</sup> *J. Inst. Brewing*, 6, 69 (1903).

<sup>7</sup> *Wochschr. Brau.*, 51, 60 (1934).

<sup>8</sup> *Ibid.*, 47, 471 (1930).

<sup>9</sup> *J. Ind. Eng. Chem.*, 15, 1074 (1923).

<sup>10</sup> *Bull. trimest. assoc. élèves école super. brasserie univ. Louvain*, 29, 30 (1929).

indefinite, due to the coloration of the sample, and losses will probably occur during the pipetting of the sample. From a practical standpoint any method requiring as a sample a portion of the contents of a bottle involves an inconvenient technic and variable carbon dioxide losses.

Baker and Hulton<sup>1</sup> add the beer to standard barium hydroxide solution and titrate with oxalic acid, using phenolphthalein as indicator. A blank determination is conducted on a boiled-out sample of the beer to correct for other substances present (sulfates, acid phosphates, weak acids, etc.), which react with the barium hydroxide. The indefiniteness of the end point and the necessity for a separate blank determination preclude this method for precise work.

There is no dearth of accurate methods for determining carbon dioxide when the material can be sampled without loss of carbon dioxide, as for example, in carbonates, baking powder, etc., and even in beer this determination by either gasometric or volumetric methods (more convenient than any present gravimetric technic) should not present any difficulty if (1) a bottle is taken as a unit charge; (2) allowance is made for handling the large volume of gas (700–1,000 cc., equivalent to 1.4–2.0 grams); (3) the evolution of carbon dioxide is controlled so as to secure a uniformly smooth liberation of gas and complete recovery is insured; and (4) the foam is suppressed.

Because of the special apparatus required and the large volume of gas to be handled, the gasometric principle was discarded by the writers in favor of the volumetric procedure. The entire bottle was used for the determination, and it was opened at extremely low temperatures and with minimum agitation. To control the rate of evolution of carbon dioxide, a small quantity of infusorial earth was used, the particles of which serve as nuclei on which carbon dioxide bubbles form as they are liberated, and in addition an efficient defoaming agent, hexyl alcohol. The carbon dioxide is evolved with the aid of heat to insure complete recovery and is absorbed in an alkaline solution, which is thereupon titrated to the phenolphthalein and methyl orange end points, use being made of a signal indicator to warn of the approach of the phenolphthalein end point. Suppressing the foam and regulating the rate of evolution make it possible to obtain highly accurate results.

The method follows:

#### CARBONIC ACID

##### REAGENT

*Sulfuric acid.*—Approximately 2.25 *N*. Standardize as follows: Weigh out 4–6 grams of anhydrous sodium carbonate, dissolve in approximately 200 cc. of water in a 500 cc. Erlenmeyer flask, and titrate to the phenolphthalein and methyl orange end points. Use the difference between these two end points to calculate the titer of the acid (about 0.1 gram of CO<sub>2</sub> per cc.).

## PROCEDURE

Remove the label and weigh the sample bottle. Cool the bottle and contents to 0° in a refrigerator or in chopped ice and allow to stand at rest overnight at this temperature.

Avoid shaking when removing the bottle from the refrigerator or chopped ice. Remove the crown and immediately add 0.1–0.2 gram of infusorial earth and 1–2 cc. of hexyl alcohol or capryl alcohol. Quickly insert a rubber stopper provided with a distilling head, the outlet end of which is provided with a rubber connection closed with a screw clamp. Clamp the bottle in position in an empty liter beaker and connect with a 250 cc. tall Drexel gas-washing bottle, which contains 25 cc. of 5 *N* potassium hydroxide solution and 150 cc. of water. The orifice of the intake tube is restricted to 1 mm. diameter at the point where the gas enters the potassium hydroxide. Open and adjust the screw clamp to permit the gas to pass into the gas-washing bottle at the rate of 3–4 bubbles per second. When the screw clamp is completely open and the gas evolution slows up, fill the beaker with water and apply the full flame of a burner. Keep the water in the beaker boiling vigorously and add boiling water to replace loss by evaporation. Continue this process until the bubbles cease to come over and the alkaline liquid in the washing bottle rises in the inner tube to the level of the outer liquid.

Disconnect the washing bottle, cool, and transfer the contents with the aid of water to a 500 cc. Erlenmeyer flask. Titrate slowly, imparting a rapid swirling motion to the contents of the flask, with 2.25 *N* sulfuric acid, using 3 drops of indicator solution (0.6 gram of thymolphthalein and 1 gram of phenolphthalein in 60 cc. of alcohol diluted with 40 cc. of water) until the lavender color of the solution changes to phenolphthalein pink. Continue to a faint pink and note the buret reading.

Then add 2 drops of methyl orange indicator (0.2 gram of methyl orange in 100 cc. of water) and continue the titration to the methyl orange end point. Correct the number of cc. of standard acid required to pass from the phenolphthalein to the methyl orange end point for the blank due to carbonates inherent in the potassium hydroxide solution, determined by titrating, in the same manner, 25 cc. of the potassium hydroxide solution in 150 cc. of water. During the titration between the two indicator changes the carbon dioxide of the beer is present in the potassium hydroxide solution as potassium bicarbonate.

Disconnect the beer bottle, wash, drain, and allow to dry. Weigh the bottle and crown. Calculate carbon dioxide as percentage by weight.

The strengths of acid and alkali chosen give convenient buret readings and yet sufficiently precise results for normal beer under ordinary conditions. A higher degree of precision, however, may be obtained by reducing the strength of the titrating acid.

It is essential to have a substantial excess of potassium hydroxide in the gas absorption bottle over the amount necessary to combine with the carbon dioxide, the amount of this excess being furnished by the titration figures. The titration to the phenolphthalein end point should always be substantially greater than one-half the total titration to the methyl orange end point.

Controls on the method were carried out by weighing a charge of sodium carbonate, dissolving in water, and transferring to a beer bottle containing infusorial earth and methyl orange indicator. The bottle was

also equipped with a dropping funnel containing sulfuric acid. The acid was allowed to drop in slowly until the sample was acid. Then the determination of carbon dioxide was carried out in the regular way. Table 1 gives the results of these tests.

TABLE 1.—*Recovery of CO<sub>2</sub> from Na<sub>2</sub>CO<sub>3</sub> solution*

| Na <sub>2</sub> CO <sub>3</sub> TAKEN | CO <sub>2</sub> TAKEN | CO <sub>2</sub> FOUND | RECOVERY        |
|---------------------------------------|-----------------------|-----------------------|-----------------|
| <i>grams</i>                          | <i>grams</i>          | <i>grams</i>          | <i>per cent</i> |
| 6.161                                 | 2.557                 | 2.540                 | 99.3            |
| 9.969                                 | 2.893                 | 2.910                 | 100.6           |
| 5.012                                 | 2.081                 | 2.035                 | 97.8            |
| 4.689                                 | 1.946                 | 1.940                 | 99.7            |
| 8.581                                 | 3.562                 | 3.540                 | 99.4            |

Strictly speaking, the results of repeated determinations on a series of bottles of beer from the same bottling are not a conclusive test of the precision of the method, since slight variations in carbon dioxide in the individual bottles may occur. Nevertheless, in Table 2 will be found the results of triplicate determinations of carbon dioxide by the proposed method on a number of beers, each sample comprising three bottles subjected separately to the determination.

TABLE 2.—*Results of determinations of CO<sub>2</sub> on beer bottled in sequence*

| SAMPLE NO. | CO <sub>2</sub> |                 |                 |
|------------|-----------------|-----------------|-----------------|
|            | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1          | 0.423           | 0.427           | 0.422           |
| 2          | 0.391           | 0.392           | 0.387           |
| 3          | 0.484           | 0.483           | 0.473           |
| 4          | 0.467           | 0.471           | 0.477           |
| 5          | 0.456           | 0.457           | 0.442           |
| 6          | 0.498           | 0.492           | 0.507           |

The concordance obtained in these results (Table 2) and also in many other tests shows that the method is highly precise.

For ordinary purposes, the extremely slight loss of carbon dioxide possibly occurring when the refrigerated bottle is opened is inconsequential, but it may be well to show how the amount lost in this way can be obtained by calculation. It is essential to know the volume of the head space and the partial pressure of the carbon dioxide, which is dependent upon the temperature and the percentage of carbon dioxide present. The head space is easily measured. The partial pressure of the carbon dioxide may be obtained with sufficient accuracy from any of the widely circulated carbon dioxide-solubility tables or by calculations from

Henry's law constant discussed later. The theoretical loss calculated in this way for four levels of carbon dioxide concentration and when 15, 20, and 25 cc., respectively, of head space exists is shown in Table 3.

TABLE 3.—*Calculated loss of CO<sub>2</sub> on opening bottle at 0° C.*

| HEAD SPACE:               |     | 15 cc.                |     | 20 cc.                |     | 25 cc.                |     |
|---------------------------|-----|-----------------------|-----|-----------------------|-----|-----------------------|-----|
| CO <sub>2</sub> IN SAMPLE |     | LOSS ON 12 OZ. BOTTLE |     | LOSS ON 12 OZ. BOTTLE |     | LOSS ON 12 OZ. BOTTLE |     |
| per cent                  | mg. | per cent              | mg. | per cent              | mg. | per cent              | mg. |
| 0.35                      | 4   | 0.001                 | 5   | 0.001                 | 7   | 0.002                 |     |
| 0.40                      | 8   | 0.002                 | 11  | 0.003                 | 13  | 0.004                 |     |
| 0.45                      | 12  | 0.003                 | 16  | 0.005                 | 20  | 0.006                 |     |
| 0.50                      | 16  | 0.005                 | 22  | 0.006                 | 27  | 0.008                 |     |

For most cases the calculated loss at 0° C. is no greater than 0.005 per cent, and even when 0.50 per cent of carbon dioxide, by weight, is present in the sample, the loss does not exceed 0.01 per cent, even when a rather large head space (25 cc.) is present.

Carbon dioxide in bottled beverages is often expressed in "volume %," which usually denotes the volume of carbon dioxide measured at some particular temperature, usually 25° C., and referred to 0° C. and 760 mm. pressure, per volume of liquid. It may be calculated from the percentage by weight figures by using 1.976 grams as the weight of one liter of carbon dioxide at 0° C. and 760 mm. pressure and correcting for the specific gravity of beer.

### PRESSURE METHODS

Pressure methods are so readily carried out when the combined crown piercing and pressure gage device is available that they would be well suited for routine control work if the two inherent errors discussed below could be corrected.

Bottled beverages contain varying amounts of air. Owing to the greater insolubility of air as compared with carbon dioxide a small amount of air represents a relatively large pressure, which the method assumes to be due to carbon dioxide, and an error is thus produced out of proportion to the amount of air usually present. The second, and lesser, error is due to the fact that solubility tables for different pressures and temperatures are based on distilled water and not on the solubility of carbon dioxide in beer or other liquids under consideration.

In Table 4 will be found the results of a number of tests of samples, arranged in the order of increasing air content, showing the comparison of the pressure method with the chemical method.

The determination of air and the pressure reading were obtained at the same time by means of the usual pressure gage-crown piercing instrument,



TABLE 4.—Comparison of results by the chemical and pressure methods

| EXPERIMENT NO. | AIR  |                        |                        | CARBON DIOXIDE            |                          |  | DEVIATIONS FROM CHEMICALLY DETERMINED CO <sub>2</sub>       |                                      |  |  |
|----------------|--|------------------------|------------------------|---------------------------|--------------------------|--|---|--------------------------------------|--|--|
|                | GAGE READINGS<br>LBS./SQ. IN.*<br>(25° C.) | PER BOTTLE<br>(25° C.) | HEAD SPACE<br>(25° C.) | DETERMINED<br>CHEMICALLY† | OBTAINED<br>FROM CHARTS‡ | CHART<br>VALUES<br>CORRECTED<br>FOR AIR‡ | ABS. PRESS.<br>COR. FOR<br>AIR IN LBS./SQ.<br>IN. X 0.00085 | CHARTS<br>UNCOR. FOR<br>AIR<br>(5-6) | CHARTS<br>AFTER COR.<br>FOR AIR<br>(7-8) | ABS. PRESS.<br>COR. FOR AIR IN<br>LBS./SQ. IN.<br>X 0.00085<br>(9-8) |
|                |  |                        |                        |                           |                          |  |   |                                      |  |  |
| (1)            | (2)  | (3)                    | (4)                    | (5)                       | (6)                      | (7)                                      | (8)   | (9)                                  | (10)                                     | (11)   |
|                |  | cc.                    | per cent               | per cent/ugl.             | per cent/ugl.            | per cent/ugl.                            | per cent/ugl.   | per cent                             | per cent                                 | per cent   |
| 1              | 27   | 1.1                    | 6                      | 0.39                      | 0.42                     | 0.41                                     | 0.39  | +0.03                                | +0.02                                    | 0.00   |
| 2              | 35.5                                       | 1.5                    | 10                     | 0.47                      | 0.50                     | 0.48                                     | 0.46  | +0.03                                | +0.01                                    | -0.01  |
| 3              | 35   | 1.6                    | 10                     | 0.47                      | 0.49                     | 0.48                                     | 0.47  | +0.02                                | +0.01                                    | 0.00   |
| 4              | 31   | 1.6                    | 8                      | 0.41                      | 0.46                     | 0.45                                     | 0.42  | +0.05                                | +0.04                                    | +0.01  |
| 5              | 34.5                                       | 1.7                    | 11                     | 0.47                      | 0.49                     | 0.48                                     | 0.45  | +0.02                                | +0.01                                    | -0.02  |
| 6              | 35   | 2.0                    | 7                      | 0.47                      | 0.49                     | 0.48                                     | 0.46  | +0.02                                | +0.01                                    | -0.01  |
| 7              | 36   | 2.0                    | 13                     | 0.47                      | 0.50                     | 0.48                                     | 0.47  | +0.03                                | +0.01                                    | 0.00   |
| 8              | 29   | 4.1                    | 20                     | 0.40                      | 0.44                     | 0.41                                     | 0.39  | +0.04                                | +0.01                                    | -0.01  |
| 9              | 43   | 4.4                    | 24                     | 0.49                      | 0.58                     | 0.54                                     | 0.51  | +0.09                                | +0.05                                    | +0.02  |
| 10             | 33   | 4.6                    | 23                     | 0.41                      | 0.48                     | 0.44                                     | 0.42  | +0.07                                | +0.03                                    | +0.01  |
| 11             | 36   | 6.0                    | 30                     | 0.43                      | 0.50                     | 0.46                                     | 0.43  | +0.07                                | +0.03                                    | 0.00   |
| 12             | 41   | 6.6                    | 31                     | 0.50                      | 0.55                     | 0.51                                     | 0.49  | +0.05                                | +0.01                                    | -0.01  |
| 13             | 31   | 7.0                    | 40                     | 0.39                      | 0.46                     | 0.40                                     | 0.38  | +0.07                                | +0.01                                    | -0.01  |
| 14             | 38   | 9.4                    | 47                     | 0.43                      | 0.52                     | 0.46                                     | 0.44  | +0.09                                | +0.03                                    | +0.01  |
| 15             | 46   | 9.8                    | 52                     | 0.50                      | 0.60                     | 0.52                                     | 0.50  | +0.10                                | +0.02                                    | 0.00   |
| 16             | 41   | 10.2                   | 57                     | 0.43                      | 0.55                     | 0.47                                     | 0.45  | +0.12                                | +0.04                                    | +0.02  |
| 17             | 31   | 10.6                   | 53                     | 0.37                      | 0.46                     | 0.38                                     | 0.36  | +0.09                                | +0.01                                    | -0.01  |
| 18             | 44   | 11.0                   | 50                     | 0.50                      | 0.58                     | 0.51                                     | 0.49  | +0.08                                | +0.01                                    | -0.01  |
| 19             | 43   | 12.8                   | 51                     | 0.47                      | 0.57                     | 0.50                                     | 0.48  | +0.10                                | +0.03                                    | +0.01  |
| 20             | 48   | 13.4                   | 67                     | 0.50                      | 0.62                     | 0.52                                     | 0.50  | +0.12                                | +0.02                                    | 0.00   |
| 21             | 40   | 14.0                   | 74                     | 0.43                      | 0.54                     | 0.44                                     | 0.42  | +0.11                                | +0.01                                    | -0.01  |
|                |  |                        |                        |                           |                          |  |   | Average                              | +0.02                                    | -0.001   |

\* Pressure above atmospheric.

† Average of at least two determinations made on companion bottles in sequence from one bottling unit—as also bottles represented in the pressure method.

‡ Carbonic acid solution, measuring charts furnishing CO<sub>2</sub> solubility data are widely distributed by various firms supplying the carbonated beverage industry with carbonic acid and carbonating equipment.

equipped with a side tube. The side tube was connected to the gas absorption buret provided with a bulb containing strong alkali to absorb the carbon dioxide. The entire system was made air free by filling the tubing with water and allowing the alkali solution to displace all air in the apparatus. After the pressure reading had been taken in the usual way, the cock leading to the absorption buret was opened, and the mixture of carbon dioxide and air was allowed to pass over into the alkali. The bottle was shaken and tapped to drive the air over. The residual unabsorbed gas was measured and considered to represent "air." Since air only was being determined, complete recovery of the carbon dioxide was unnecessary. About 90 per cent of the measured air came over with the first 100 cc. of carbon dioxide. The operation of tapping the bottle and shaking the absorption buret bulb was repeated until the reading of the volume of air in the graduated portion of the tube was constant.

Both cubic centimeters of air per bottle and its percentage of the head space present in each bottle are given in Table 4.

The percentage by weight (column 6) was calculated from the volume percentage furnished in one of the widely used solubility pressure tables. It will be seen (columns 5 and 6) that the carbon dioxide obtained from pressure readings deviates (calculated in column 9) from the chemically determined carbon dioxide results by varying amounts, depending upon air content, ranging from about 0.02 per cent, where the air content is low, to 0.12 per cent, representing the rather serious error of more than 25 per cent, when the air content is high. Such air contents in bottled beer (74 per cent of the head space) are not unusual.

Having determined the percentage of air in the head space, the analyst can readily adjust the error in the pressure method from this source. The partial pressure of the air is proportional to the amount present. It is then only necessary to multiply 14.7 (one atmosphere) by the decimal fraction representing the percentage of air in the head space to obtain the partial pressure due to air (50 per cent air would involve a partial pressure of 0.50 of 14.7 or 7.35 lbs., etc.). The total gage pressure reading, less the partial pressure thus found, is the pressure due only to the carbon dioxide. The values listed in column 7 were calculated from the same charts as was column 6, except that the pressure readings were corrected for the amount of air present in this way. The uniformly closer agreement in the results with the chemically determined values is apparent.

With regard to the lesser error, it is necessary to refer to the theoretical background of the pressure method, namely Henry's law, which states that the solubility of a gas in a liquid is directly proportional to the partial pressure of the gas. It is usually expressed as follows:

$$K = \frac{P}{X}, \text{ where}$$

$K$  equals the Henry's law constant for the particular gas,  $P$  equals the partial pressure of the gas in mm. of mercury (760 mm. at one atmosphere pressure), and  $X$  represents the mol fraction of the gas in solution. Since  $X$  represents a mol fraction of dissolved carbon dioxide, that is mols carbon dioxide per mol solution, by converting the pressure expressed in millimeters of mercury to pressure in pounds per square inch, there can be obtained from the published value of  $K$  a value " $a$ ," representing weight percentage of carbon dioxide for each pound of absolute partial pressure due to carbon dioxide. Thus

$$X = \frac{\text{Mol CO}_2^*}{\text{Mols water}} \text{ or } \frac{A/44}{100/18}.$$

By substituting in the Henry's law equation above this equivalent for  $X$  and the following values:  $p=760$  mm. (one atmosphere), and  $K=1.243 \times 10^6$  (at  $25^\circ \text{C.}$ )<sup>1</sup> and solving for " $A$ ," which represents grams of carbon dioxide per 100 grams of water at 760 mm. pressure and at this temperature ( $25^\circ \text{C.}$ ), the value 0.1494 is obtained. This value divided by 14.7 gives the desired " $a$ " at  $25^\circ \text{C.}$  as 0.0102.

The commonly used charts are based on the published values of  $K$  as given in Table 5. Calculations of " $a$ " from random points along the  $25^\circ \text{C.}$  line on such a chart hence yield the identical value, 0.0102. Thus the results obtained in columns 6 and 7 (Table 4) from such charts are identical with results obtainable by calculation from the gage pressure, using this factor, 0.0102, as " $a$ ."

If it is assumed that in beer the presence of the small quantity of alcohol does not appreciably change the solvent characteristic of the water and that the extractives act only to reduce the quantity of solvent available, it may be considered that for an average beer the solvent content will be about 94 per cent, rather than 100 per cent.  $X$  then becomes

$$X = \frac{A/44}{94/18}.$$

By proceeding as directed above the value 0.0095 is obtained for " $a$ " at  $25^\circ \text{C.}$

It is believed that this value more nearly fits the conditions for beer than does the factor 0.0102. These deductions necessarily apply equally to temperatures other than  $25^\circ \text{C.}$  provided the appropriate value of  $K$  corresponding to the particular temperature is employed.

In column 8, Table 4, are shown the results obtained by calculation from the pressure readings after correcting for air, as described, by the

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\* Theoretically this should be:  $\frac{\text{Mol CO}_2}{\text{Mols H}_2\text{O} + \text{Mols CO}}$ , but no appreciable error is involved in the simplification.

<sup>1</sup> International Critical Tables, Vol. III.

factor 0.0095 instead of consultation of the charts. It should be remembered that this factor "a" represents the weight per cent of carbon dioxide per pound absolute partial pressure and hence 14.7 lbs. (one atmosphere) must be added to the gage reading (after correcting for air) before multiplying by "a."

In columns 10 and 11 are given the deviations from the chemically determined results, represented by the results obtained in both cases after correcting for air, by using (1) one of the commonly used charts (column 7), ("a" at 25° C. = 0.0102), and (2) the factor determined by calculation from an assumed average beer ("a" at 25° C. = 0.0095) (column 8).

It will be seen that the results from chart data run slightly higher than the chemical results, while the factor of 0.0095 yields results in very close agreement with the chemically determined values. On the average there is a deviation of +0.02 per cent for the chart values, whereas the similar average for the calculated values is -0.001 per cent.

Hartung<sup>1</sup> recently published complete graphs and tables based on an exhaustive series of experiments, in which a large number of samples collected under special conditions from tanks of beer in a brewery were carefully tested, both as to chemical carbon dioxide content and pressure. His figures show that the values ("a") as calculated from a number of his points at random at varying temperatures correspond closely with the factor "a" as calculated in this paper. Table 5 makes this point clear.

TABLE 5.—Increment of CO<sub>2</sub> "a" per lb. partial pressure

| TEMP. | KX11 <sup>-60</sup> | FROM TABLES IN<br>COMMON USE BASED<br>ON 100% WATER | CALCULATED FROM<br>HARTUNG'S<br>EXPERIMENTAL<br>RESULTS | CALCULATED ON<br>ASSUMPTION<br>OF 94%<br>"WATER" |
|-------|---------------------|---|---|--|
| °C.   |                     | per cent  | per cent  | per cent   |
| 0     | 0.553               | 0.0220  | 0.0217  | 0.0215   |
| 5     | 0.666               | 0.0191  | 0.0180  | 0.0178   |
| 10    | 0.792               | 0.0160  | 0.0153  | 0.0150   |
| 15    | 0.930               | 0.0137  | 0.0132  | 0.0128   |
| 25    | 1.243               | 0.0102  | —   | 0.0095   |

\* International Critical Tables, Vol. III.

### SUMMARY

(1) A simple, rapid, and precise chemical method for determining carbon dioxide in bottled beer, applicable also to other carbonated beverages, is described.

(2) This method yields results within 0.01 per cent in a sequence of bottles from the same bottling.

(3) With the aid of the chemical method developed a study was made of the customary pressure method for determining carbon dioxide in beer and carbonated beverages.

<sup>1</sup> Communications, Master Brewers Assoc. of America, Vol. 3, No. 3 AR, p. 4. Sept. 1934.

(4) The pressure method is shown to have two sources of error: (a) air, and (b) the fact that tables used are based on the solubility of carbon dioxide in water.

(5) A method is described for correcting the error introduced by the "air" in determining carbon dioxide by the customary pressure method.

(6) The difference between tables based on solubility of carbon dioxide in water and factors based on its solubility in beer is shown.

(7) Correction for air and the use of revised solubility values in pressure methods calculated for average beer are recommended.

## BOOK REVIEWS

**Essentials of Physiological Chemistry.** By ARTHUR K. ANDERSON. 257 pages, 31 illustrations. John Wiley and Sons, Inc., New York, 1935. Price \$2.75.

This text, which is designed primarily for students of home economics, agriculture, and dentistry; premedical and veterinary students; and for students of physiological chemistry in small colleges, the reviewer considers admirably suited for this purpose. Although much of the subject matter is of a controversial nature, the writer has been sufficiently dogmatic to avoid leaving the beginning student merely in a state of confusion. The reviewer was particularly struck with the meticulous care the author has evidently used to avoid typographical errors, admittedly inexcusable, but one of the most common faults of first editions. Their scarcity made it a pleasure to read this text.

There are, however, a few possible criticisms that may be mentioned. There is a typographical error on page 45, where an "H" is omitted in the formula of fructose, and on page 210 the double bond in the formula for adrenalin should be a single bond. It seems rather odd that the old formulas for cholesterol and guanylic acid should be given instead of the new, when such formulas as those for pectic acid, vitamin B, and ergosterol are included. Again it is to be regretted that the highly speculative theory of Krebs for urea formation should be included and the thrilling work of Rose on the leucines, phenylalanine, and the new "essential factor" should be omitted. A hard question for the author to explain may be that put by the alert student when he notices the difference between the formula for hydroxyproline given on page 85 and that given on page 88. There also seems to be no evidence for the statement on page 114 that the gastro-intestinal enzymes may be responsible for the synthesis of amino acids into tissue proteins. The two systems of enzymes are entirely different in properties. Finally, omitting a few other minor things, a strong plea should be made for a more complete index. For example, to mention only a few instances, "benzene," "turpentine," "aniline," "bromellin," "keratin," "elastin" are terms used in the context, but they are not included in the index, while "butter-fat" is referred to on page 69 but not on page 73, where a very important property of butter-fat is recorded.

All these instances, however, relate to minor matters that can be corrected in a subsequent printing. The simplicity of style and comprehensiveness will, no doubt, make the book a popular introductory text.—HERBERT O. CALVERY.

**Dictionary of Terms Relating to Agriculture, Horticulture, Forestry, Cattle Breeding, Dairy Industry, and Apiculture in English, French, German, and Dutch,** compiled by T. J. BEZEMER. Pp. VII+1062. Williams & Wilkins Co., Baltimore. Price \$8.00.

This book is not a descriptive or defining dictionary, but an attempt to provide equivalents in four languages of about 10,000 of the most important terms used in agriculture and related fields. In section 1, Dutch terms are listed alphabetically with their one- or two-word equivalents in German, English, and French. Sections 2, 3, and 4 provide a rearrangement of these terms based on the three other languages in turn. This method results in a book of more than 1,000 pages, but the size (5½ by 8½ by 2 in.) is easily manageable, and the type is exceptionally clear and readable for a work of this kind.

The compilation was made at the State Agricultural College at Wageningen, Netherlands, where the editor is professor of languages. About 30 collaborators, located mostly at Wageningen or at other Netherland institutions, assisted in the task. On this staff most of the various fields of agriculture were represented, and

the work as a whole seems well balanced and as comprehensive as would be expected within the space limits available. Indicating as it does many specialised meanings which have been covered inadequately, if at all, in general translating dictionaries, the book should prove to be a very useful supplementary aid to students and others who have occasion to consult foreign agricultural literature.—HOWARD LAWTON KNIGHT.

**Structure and Properties of Matter.** By HERMAN T. BRISCOE. 420 pages. Illustrated. McGraw-Hill Book Co., New York, 1935. Price \$3.75.

This book is a review of the physical concepts of atomic structure and some of the interpretations of physical and chemical phenomena resulting from these concepts. The author presents in a narrative style the development of present-day theories concerning the structure of the atoms. The reader should bear this philosophic presentation in mind throughout the book. It makes the subject matter seem more substantial than a picture drawn from the viewpoint of the experimentalist. The bibliography includes books rather than current professional literature, and this form aids in making coherent the philosophies developed. It is to be regretted that the position of energy concepts is implied rather than stated since a fundamental key to the development of theory and its application to practical work is thus neglected.

To those workers taught the art of evaluation by experience, and whose work has long been in terms of practical use, this book will bring a story of recent thought in atomistics perhaps not formerly available.—R. U. BONNAR.







JAMES MONROE BARTLETT, 1856-1935

## JAMES MONROE BARTLETT

"Holmes Hall, headquarters of the Maine Agricultural Experiment Station, was closed the afternoon of May thirteenth. Flowers and a gray ribbon hung as a symbol upon the locked door. James Monroe Bartlett may no longer be seen in the laboratory over which he presided for a full half century.

"Yet a sense of his presence remains in that room. It lingers as an echo of quiet counsel spoken to those who came to him seeking advice in matters of importance. It stays, as a savor of gentle mirth, in the recollection of those with whom he shared his whimsical perspectives. His associates find comfort in their experience that they need not lose such impressions of companionship.

"There is, indeed, solace in the knowledge that the attributes of those we honor are not taken from us but are bestowed like gifts we may retain in grateful memory. Generous is the endowment we have thus received from Doctor Bartlett. If we hold the fullness of our appreciation of his kindness and wisdom in silent thought, it is because we know that our friend would prefer such unspoken tribute.

"It may not be out of place to remark that the members of the Station Staff are glad that expressions of affectionate esteem were not delayed until the hour of obituary. Last September we met to celebrate the eightieth birthday anniversary of our colleague. April 29 some four-score of his friends gathered about banquet tables to rejoice that he had been a member of the Staff for fifty years. In a room made beautiful by flowers, with gracious music, with friendly reminiscence, and with gifts (a golden watch and fifty roses) homage was rendered to him and to his wife. The sentiment of that evening is good to recall as a part of our happy association with James Monroe Bartlett—true in personal friendships and true in the tasks of his long and notable service in the field of science."

Thus did Dr. Edith M. Patch, a co-worker in the Maine Station for over a quarter of a century, pay tribute to Dr. Bartlett in "The Maine Campus" of May 17, 1935.

Dr. Bartlett's boyhood was spent on a farm in Litchfield, Maine, where he was born September 25, 1856, the son of James and Sarah (Towne) Bartlett. After attending the Academy in his native town he entered the Maine State College, in 1876, and was graduated four years later with the degree of B.S. in Chemistry. Following graduate work at Cornell University he received his Master's Degree from Maine in 1883 and accepted a position as Assistant Chemist at Pennsylvania State College, where he remained two years.

On May 1, 1885, he returned to his native State as a member of the staff of the Maine Fertilizer Control and Agricultural Experiment Station, first as Assistant Chemist and soon as Chief Chemist, which latter position he held until his death, May 11, 1935. His wife, who survives him, was Sarah Caroline Pattangall, sister of the former Chief Justice of Maine, William R. Pattangall. He is also survived by two sons, three daughters, and seven grandchildren.

No one could be associated long with Dr. Bartlett without coming to have for him keen admiration, sincere respect, and real love. Un-

assuming almost to a fault, the casual acquaintance would hardly suspect that back of the quiet exterior was a fund of expert knowledge, general information, and dry humor that few possess. Those who worked with him in the laboratory were early impressed with two characteristics. Thorough grounding in the fundamentals of analytical chemistry coupled with rare patience and ingenuity enabled him not only to arrive at correct methods of solution when problems were presented but to construct the necessary apparatus for conducting any new and unusual work; and any professional information or knowledge which he possessed was always freely given to those who worked under his direction.

His judgment was never hasty and was almost invariably sound. He was a keen observer, careful and thorough in his work, and in the interpretation of the results of a complicated analysis his conclusions were usually correct. His advice, sought continually on the most divergent topics, was always practical and has had wide influence on the agriculture of the State. While a large part of his experiment station activities was confined to the regulatory control of fertilizers, feeds, insecticides, foods, and drugs, he found time to conduct numerous research projects pertinent to the agriculture of his native state. His experiments in cattle feeding for milk production and for growth, and the digestion experiments which he conducted with sheep, steers, and poultry added materially to the knowledge of farm feeding and contributed to science valuable data on the coefficients of digestibility of many feeding materials; his investigation of the adaptability of potato varieties in Aroostook County and the methods of planting, spraying, and dusting of this important crop was instrumental in increasing materially the potato production of this section. Compared with many workers of this period he published little research under his own name for he never courted publicity or sought fame, but during the fifty years of his service most of the bulletins and reports of the Station contained valuable results of his painstaking work.

On two occasions Dr. Bartlett served as Acting Director of the Maine Experiment Station, and he was offered the post of Director. This position he declined, preferring to remain as Chemist rather than to take on new responsibilities at a time of life when he felt that he should be relinquishing rather than assuming new duties.

In 1927 the University of Maine conferred upon him the Honorary Degree of Doctor of Science as "a loyal son of the State of Maine, contributor to the scientific advancement of agriculture and contributor to important methods of analysis of the Official Agricultural Chemists Association."

Dr. Bartlett took an active interest in the work of the Association of Official Agricultural Chemists from the time of his first attendance at the 8th Annual Meeting in 1891. Although not a constant attendant he participated in the work of twenty of its meetings. At the 1894 and 1895 meetings he was Reporter on Nitrogen and at the 1908 and 1909 meetings Referee on Dairy Products. He was a member of the Executive Committee in 1910. At the 1911 meeting he served as Chairman of the Auditing Committee and at the 1913 meeting as Chairman of the Committee on Resolutions. In 1917 he was the Associate Referee on Tea and Coffee. Dr. Bartlett's most conspicuous work for the Association was the formulating of a practical and accurate method, which was

adopted as official, for determining borax in fertilizers at a time when some of the domestic supplies of potash were contaminated with this impurity. This work is comprised in his paper, "The Distillation Method for the Estimation of Borax in Mixed Fertilizers," published in the Proceedings for 1920, and in his report on "Borax in Fertilizers" for the 1922 and 1923 meetings. His obituary of Dr. Charles D. Woods was published in the Journal of the Association for August, 1926.

Dr. Bartlett exercised a great influence at the meetings of this Association not only by his papers and reports but by his constant helpful advice upon a large number of the referee projects in which he served as collaborator. He was a conspicuous figure at the semi-centennial anniversary of the Association in 1934, and the many members who met him then for the last time will long retain the memory of his kindly genial personality.

Dr. Bartlett was a member of the American Chemical Society and of the Phi Gamma Delta Fraternity. He was an ardent sportsman, and his vacations were usually taken in the woods with dog and gun or on some favorite trout stream, and on these excursions he was a delightful companion as the writer can testify from many happy memories.

With his death the community has lost a loved neighbor, the State a valued citizen, and his professional associates throughout the country an honored co-worker.

HERMAN H. HANSON

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## MONDAY—MORNING SESSION

### REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The change in the legal status of alcoholic beverages caused by the recent repeal of the 18th amendment again brought these beverages within the jurisdiction of food and drug officials throughout the nation. The Association gave little attention to its methods for the analysis of these products during the time of prohibition, but now it seems necessary to bring these methods up to date as far as possible in order that the forthcoming edition of the *Book of Methods* may contain a comprehensive chapter on this subject. In addition to the usual number of associate referees on the various divisions of the subject into which it falls naturally, namely, beers, wines, and distilled liquors, several other associate refereeships were included this year to cover specific determinations, such as fusel oil and methyl alcohol, as well as an Associate Referee on Cordials and Liqueurs, a subject which had not been studied previously by the Association.

#### BEERS

While J. A. LeClerc did not directly do any collaborative work on this subject during the year, he consulted with numerous commercial and brewing chemists and is recommending certain changes in the methods for beers, with the result that they will be modernized and brought into line with the methods in use by brewers throughout the country. In his opinion, sufficient collaborative work has been done upon these methods to warrant their inclusion in the new edition of *Methods of Analysis*.

#### WINES

No collaborative work was done by B. G. Hartmann on this subject, but he corresponded with wine chemists throughout the country and received comments from them regarding the methods in the 1930 edition. Certain additional changes which do not affect the underlying principles of the methods are being made. Methods for the determination of fruit acids are being added, and a program of work has been outlined to deal with the three broad types of adulteration of wine, namely, the use of pomace extract, the addition of water, and the addition of other fruit juices, such as apple juice, to grape must.

#### CORDIALS AND LIQUEURS

John B. Wilson, the associate referee on this subject, submitted a group of tentative methods for the analysis of liqueurs and cordials which,

while they do not permit the determination of all of the many constituents that may be present in cordials, do offer a basis upon which to judge their composition. No such methods have been included in previous editions of *Methods of Analysis*. Collaborative work was done on methods for the determination of benzaldehyde, gamma-undecalactone, and volatile esters. Study of volatile esters was necessary since the large proportion of solid matter in cordials renders the official method for esters in distilled liqueurs inapplicable.

#### WHISKEY, RUM AND BRANDY

G. F. Beyer answered certain criticisms of the present methods for these products. He recommends a few minor revisions of the present chapter.

#### FUSEL OIL

Peter Valaer traced the history of the present method for the determination of fusel oil within and without the Association. While no collaborative results are presented, a study of the various stages of the method was made, and its retention is recommended.

#### METHYL ALCOHOL

During the past year a method for the determination of small quantities of methyl alcohol in the presence of relatively large proportions of ethyl alcohol was published by John B. Wilson, *This Journal*, 18, 477 (1935). E. M. Bailey, Associate Referee on Methyl Alcohol, submitted the method to collaborative work, and it is now recommended that with certain minor changes recommended by the associate referee the method be adopted as a tentative method, with a view to doing further collaborative work to establish whether or not this method should be made official. At the same time the present colorimetric method for methyl alcohol is recommended for retention as a tentative method.

The referee approves of the recommendations presented by the associate referees and also the changes in the alcohol tables published in *This Journal*, 19, 75 (1936).

#### REPORT ON BEERS

By J. A. LECLERC (Bureau of Chemistry and Soils,  
Washington, D. C.), *Associate Referee*

The methods of the A.O.A.C. for the analysis of beers have remained essentially without change since the "Official and Provisional Methods of Analysis" of this Association was issued nearly 30 years ago.

The following recommendations are made:<sup>1</sup>

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<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 76 (1936).

(1) Retain with slight or no change the following methods (numbers refer to chapter XVII, *Methods of Analysis*, A.O.A.C., 1930):

(1) Preparation of sample, Official; (4) Alcohol, Official; (6) Method II, Extract by Immersion Refractometer, Tentative; (7) Extract (Method III by calculation), Official; (10) Total Acids, Official; (11) Volatile Acids, Official; (12) Reducing Sugars, Official; (13) Dextrin, Tentative; (14) Direct Polarization, Tentative; (15) Glycerol, Official; (16) Ash, Official; (17) Phosphoric Acid, Official; (18) Protein, Official; (19) Preservatives, Official; (20) Coloring Matters, Tentative; (21) Metals, Tentative.

(2) Make the following changes in methods:

(2) Color, Tentative—use a “1/2” instead of a “1/4” cell; (3) Specific Gravity, Official—determine the specific gravity in air at 20/20°, instead of at 20/4°.

(3) Delete the following methods:

(5) Extract (Method I), Official; (8) Extract of Original Wort, Official; and (9) Degree of Fermentation, Official (first action).

(4) Adopt the following methods as tentative:

Apparent Extract—ascertained from Table 2 (Modified); Real Extract; Extract of Original Wort; Apparent Degree of Fermentation, Calculated; Real Degree of Fermentation; Fermented and Unfermented Extract; H-ion concentration; Carbonic Acid (Gray-Stone method); Sulfurous Acid; Iodine Reaction; Pasteurization; Chlorides; Methyl Alcohol.

Modify Table 2 under the direction of the associate referee.

Insert table, “Comparison of Balling and Plato Tables.”

It is suggested that the title of the chapter carrying these methods be, “Malt Beverages, Extracts and Sirups, and Brewing Materials.”

The thanks of the associate referee are hereby given to H. W. Rohde for his most helpful cooperation and to various members of the M.A.S.C. for their help and suggestions.

A paper, entitled “The Determination of the Peptic Power of Malt and Its Value in Brewing,”<sup>1</sup> was presented by Arnold Spencer Wahl, Chicago, Ill.

A paper, entitled “Methods of Determining Carbon Dioxide in Beer and Carbonated Beverages,” by Philip P. Gray and Irwin M. Stone, New York City, was presented. This paper was published in *This Journal*, 19, 162 (1936).

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<sup>1</sup> To be published in the May number of the American Brewers' Review.



## REPORT ON WINES

By B. G. HARTMANN (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Considerable time was devoted to the revision of the chapter on Wines in *Methods of Analysis*, 1930. Before undertaking the work the associate referee requested an expression of opinion from interested chemists regarding desirable changes in the present methods.

Reports were received from the following chemists:

- C. S. Ash, California Packing Corp., San Francisco, Calif.
- E. M. Bailey, Agr. Exp. Sta., New Haven, Conn.
- M. P. Duffy, State Dept. of Public Health, San Francisco, Calif.
- H. E. Goresline, Bur. Chemistry and Soils, Washington, D.C.
- A. A. Jackson, Schwarz Laboratories, New York
- M. A. Joslyn, University of California, Berkeley, Calif.
- W. Siebenberg, Schwarz Laboratories, New York

The associate referee takes this opportunity to thank these chemists for their suggestions, and, realizing the value of their contributions to future investigations on the subject of wine analysis, is presenting a brief resumé of the several reports. The numbers refer to the items under Wines in Chapter XVII, *Methods of Analysis*, 1930.

22. Joslyn suggests microscopic examination for acetic, lactic, and Tourné bacteria. For detecting off odors he suggests rubbing a few drops of wine between the palms of the hands and holding them cupped before the nose.

Duffy thinks color classification (sub 4) is vague, also that chemists are not qualified to pass on sub 5 and 6.

23. Duffy suggests filtering as an alternative for degassing wines.

Joslyn suggests immediate determination of tartaric acid and tannins.

25. Duffy believes immersion refractometer for alcohol is not accurate enough for official work.\*

Joslyn suggests ebullioscope for official work.

26. Joslyn suggests deletion.

Duffy believes method sufficiently accurate.

Jackson believes ashing temperature of the isolated glycerine should be controlled.\*

27, 28, 29. Joslyn suggests deletion.

30. Joslyn believes procedure satisfactory for regulatory purposes. He, however, suggests consideration of other densimetric methods (Ackerman, Roussopoulos Dujardin-Salleron, de Houdart).\*

Ash recommends consideration of refractometric methods on the dealcoholized wine.\*

Duffy believes procedure satisfactory. However, he suggests changing the formula to read  $S = G - A + 1$ .

31. Duffy suggests refractive index of the dealcoholized wine.\*

33. Duffy prefers the modified Brown-Morris-Miller method.

Goresline recommends study of the colorimetric method of Poe and Edson.

Joslyn finds Lane-Eynon method satisfactory. He suggests study of clarification with charcoal or mercury.\*

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\* Items considered of sufficient importance to warrant study, which is recommended.

34. Duffy believes determination useless since all sugars would be inverted after a few months.

Joslyn prefers inversion in cold and suggests volumetric method.

37. Siebenberg suggests temperature control.

42. Joslyn suggests addition of  $H_2SO_4$  and reignition to convert  $BaS$  into  $BaSO_4$ .

43. Joslyn suggests deletion.

44. Duffy, Goresline, and Siebenberg suggest electrometric titration.

In the case of white wines Joslyn suggests diluting 10 cc. of wine with 200 cc. of boiling  $H_2O$  and the use of phenolphthalein as indicator. For red wines he suggests consideration of decolorizing with charcoal or the use of smaller volumes (micro buret).<sup>\*</sup> Suggests incorporation of lead acetate method.

45. Duffy suggests distillation without steam.

46. Duffy believes not satisfactory.

48. Joslyn suggests study of pH effect on tartaric acid recovery.

50, 51. Joslyn and Duffy suggest zinc salt to precipitate tannin instead of removal with bone-black.<sup>\*</sup>

53. Joslyn suggests deletion.

54. Duffy suggests test for pectin.

55. Joslyn suggests deletion.

57. Joslyn recommends a general fermentation test for preservatives.

In addition to the above comments, E. M. Bailey suggests investigation of the Pucher-Vickery-Wakeman method for determination of acidity.<sup>\*</sup> He also believes that a determination of methyl alcohol might be of service in detecting the use of methyl alcohol denaturants. He calls attention to false salicylic acid, a volatile substance which has been found in a brand of malt extract and which with  $FeCl_3$  gives a reaction somewhat similar to that of salicylic acid.

Joslyn suggests incorporation of methods for the determination of malic, citric, and succinic acids. He also believes that methods for the determination of the degree of oxidation and esterification of wines as an index of natural or artificial age would be of value. He recommends a quantitative determination of the color of a wine.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That editorial changes which do not alter underlying principles be made in the text of some of the procedures.

(2) That in accordance with Note 9, page XVII, the temperature  $\frac{20^\circ}{4}$  in the specific gravity determination be changed to  $\frac{15.6^\circ}{15.6}$ ,  $\frac{20^\circ}{20}$ ,  $\frac{25^\circ}{25}$ .

(3) That the caption "Total Acids" be changed to "Acidity." The term "Total Acids" includes both free and combined acid constituents, whereas titration represents only the free acidity (titratable).

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 78 (1936).

(4) That in addition to the methods described under total acids, titration of the highly diluted wine with phenolphthalein be included.

(5) That in the determination Volatile Acidity provision for correction for  $\text{SO}_2$  be made and be inserted under the heading, "Volatile Acidity Exclusive of  $\text{SO}_2$ ."

(6) That since the procedures for citric and malic acids described in the chapter on Fruits and Fruit Products are applicable to wines, they be incorporated in the chapter on Wines by cross reference. These acids occur in normal wines in small quantities only, and therefore for their determination 100 cc. of wine should be used.

(7) That the applicability of the method outlined for white grape juice, *Methods of Analysis*, 1930, p. 276, to the determination of added (excess) water in wines be studied.

(8) That the Werder sorbitol procedure<sup>1</sup> for determining non-grape material in wines be studied.

(9) That the applicability of the Wilson methyl alcohol determination, (*This Journal*, 18, 482 (1935), as a possible means of detecting the use of pomace in wines be studied.

(10) That the method outlined in the report of the Referee on Fruits and Fruit Products for the determination of lactic acid in wines be subjected to collaborative study.

(11) That the following procedure for the saponification of esters (required in the determination of organic acids) be studied:

After adding the required quantity of lead acetate to the depectinized sample reflux the mixture in boiling  $\text{H}_2\text{O}$  for 30 min. Cool the mixture, transfer to a centrifuge bottle with alcohol, and centrifuge.

A paper, entitled "A Method of Correcting for the Presence of Sulfurous Acid in the Determination of the Volatile Acidity of Wines," was presented by Victor de F. Henriques, Lodi, Calif.

## REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

A greater variety of composition probably exists in cordials and liqueurs than in any other class of alcoholic beverages. Their alcohol content may vary from 18 per cent by volume, as in the case of vermouth,

<sup>1</sup> *Mitt. Lebensm. Hyg.*, 20, 7-14 (1928).

to the 65 per cent by volume in many absinths. The sugar content may vary from one per cent or less, as in dry vermouth, to 46 per cent, the content often found in superfine crème de menthe or crème de curaçao.

Cordials may contain tartrates and malates, for example vermouth and other products with a wine base, or citrates and malates, as in the case of apricot and peach cordials. Again, malic acid alone may be present, as in cherry cordial, citric acid alone as in the case of crème de cassis or curaçao, or iso-citric acid, for which no method is now available, as in blackberry cordial.

The flavor of the cordial may be due in whole or in part to one or more of the following parts of the plants:

*Bark:* cascarilla or cinnamon.

*Beans:* vanilla, cacao, coffee, etc.

*Berries:* blackberries, juniper, sloe, etc.

*Buds:* cloves.

*Flowers:* violets, roses, orange blossoms, etc.

*Fruit juices:* black currants, cherries, strawberries, etc.

*Fruit peel:* bitter orange, sweet orange, lemon, etc.

*Leaves:* peppermint, bay, laurel, etc.

*Nuts:* almonds, chestnuts, walnuts, etc.

*Plant tops:* absinth, hyssop, thyme, etc.

*Roots:* angelica, orris, sassafras, etc.

*Seeds:* anise, caraway, coriander, etc.

For many years some manufacturers of cordials have compounded their products from essential oils derived from the plant materials enumerated above in order to obviate the necessity of percolating or distilling the plant material and since repeal there is also a growing tendency to use the so-called true fruit flavors, thus further restricting the equipment needed for the manufacture of cordials.

The alcohol content of the cordial may be due to the use of wine, as in vermouth and other medicinal wines; to whiskey as in cocktails; to rock and rye; or to brandy, grain alcohol, or molasses alcohol. The brandy may be made from a mash of grapes or of other fruits, such as blackberries, peaches, etc.

The regulatory chemist may be called upon to examine samples for the presence or absence of any one of the ingredients mentioned or to establish the presence of artificial color, artificial flavor, added acid, or some abnormal ingredient.

At present the associate referee is unable to submit a set of approved methods to enable regulatory chemists to make complete examinations of cordials. However, through the use of the methods submitted, which were published in *This Journal*, 19, 78 (1936), the analyst may establish the presence or absence of several of the more important constituents.

The physical examination of the sample is important because it not only assists in deciding whether or not the sample is true to type as labeled, but also because it gives an indication of the presence of artificial color, artificial flavor, essential oils, fruit juice flavor, plant extractives, etc.

The nature and quantity of sweetening ingredients is disclosed by the determination of total solids, glycerol, sucrose, and reducing sugars. The presence of fruit juices may be established by the relation between the quantities of the characteristic acids of the fruit in question and the ash, its alkalinity, and/or phosphoric acid content.

The nature of the alcoholic ingredient may be shown by determinations of esters, fusel oil, etc., unless these constituents have been added in the form of artificial flavor or essential oils.

For the most part, the methods recommended are similar to methods now having official status for use on other food products. In most cases the modifications are only such as are necessary in the preparation of the sample or in regulating the quantity of sample taken. However, in two cases the methods are sufficiently modified to require some collaborative work, and in a third case an entirely new method is given.

#### BENZALDEHYDE

The proposed method for the determination of benzaldehyde was investigated by Woodman and Davis<sup>1</sup> and found to recover about 95 per cent of the quantity added when from 5 to 81 mg. was present. The associate referee distilled phenylhydrazine at the pressure of about 60 mm., kept the distillate frozen in a refrigerator, and found that even after three months when the reagent was added to dilute acetic acid, it dissolved completely, producing a solution which was only slightly cloudy. After being filtered, the solution had a faint yellow tinge which increased rapidly on standing in the laboratory.

A solution of benzaldehyde was prepared by dissolving 4.02 grams of oil of bitter almonds in alcohol and diluting to 200 cc. so that the final product contained about 30 per cent alcohol (Soln. A); 50 cc. of Solution A was diluted to 500 cc. with 30 per cent alcohol (Soln. B).

Benzaldehyde was then determined in each solution by the tentative method for almond extract (*Methods of Analysis*, 1930, p. 289, 50).

Determinations were also made by the following modified procedure.

Pipet 10 cc. of solution (A or B) into a 300 cc. Erlenmeyer flask; add 200 cc. of distilled water, 10 or 15 cc. of phenylhydrazine reagent prepared as in the tentative method; shake for 5 minutes; filter on a tared Gooch; and wash with water until all the precipitate has collected on the mat. Now wash with two 10 cc. portions of alcohol, 10% by volume, and dry at 70° C. in a vacuum oven. Weight of precipitate  $\times 0.5408$  = benzaldehyde.

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<sup>1</sup> *J. Ind. Eng. Chem.*, 4, 588 (1912).

The results obtained are shown in Table 1.

TABLE 1.—*Benzaldehyde in Solutions A and B*

| SOLUTION | REAGENT | TENTATIVE METHOD | MODIFIED METHOD |
|----------|---------|------------------|-----------------|
|          | cc.     | gram             | gram            |
| A        | 10      | 0.1959           | 0.1954          |
| A        | 15      | 0.1978           | 0.1958          |
| B        | 10      | 0.0198           | 0.0196          |
| B        | 15      | 0.0203           | 0.0194          |

The precipitates obtained by the tentative method were darker in color than those obtained by the modified method and contained small brown specks, which were not found in the other precipitate.

Two imitation cherry cordials were then prepared for collaborative work by placing 100 grams of sugar, 0.5 gram of citric acid, and 100 cc. of water in each of two 500 cc. volumetric flasks. When the solid matter had completely dissolved, 160 cc. of alcohol was added to each flask. Different quantities of solution B were then added, and the volumes were completed to 500 cc. Cordial "C" contained 25 cc. of solution B, equivalent to 49 mg. of benzaldehyde in 500 cc., while Cordial "D" contained 100 cc. of solution B, equivalent to 196 mg. of benzaldehyde as determined by the modified procedure.

These cordials were then examined by W. O. Winkler, Washington, D. C., and the associate referee by the proposed method, with the results given in Table 2.

TABLE 2.—*Benzaldehyde in cordials by proposed method*

|           | PRESENT | WILSON | FOUND<br>WINKLER |
|-----------|---------|--------|------------------|
|           | mg.     | mg.    | mg.              |
| Cordial C | 9.8     | 9.6    | 9.1              |
| Cordial D | 39.0    | 40.6   | 38.0             |

#### GAMMA-UNDECALACTONE

The procedure for the detection of gamma-undecalactone, *This Journal*, 19, 80 (1936), was submitted for collaborative work, as far as the separation of this substance from a synthetic cordial, to three analysts. However, as only one microanalyst in the Food and Drug Administration is qualified to identify the crystals under the microscope by the immersion method, it was necessary that each of the analysts submit the crystals of hydrazino-gamma-undecalactone to G. L. Keenan, Micro-analytical Division, for identification, after separating them from the sample. In order that the sample would contain as many as possible of the types of substances which might interfere with the detection of this

substance, the synthetic cordial was made containing the ingredients listed below:

|                        |                                 |
|------------------------|---------------------------------|
| Sugar—600 g.           | Benzaldehyde—0.10 g.            |
| Alcohol—750 cc.        | Gamma-undecalactone—0.106 g.    |
| Vanillin—0.06 g.       | Citric acid—8.0 g.              |
| Caprylic acid—0.07 g.  | Caramel color—quant. sufficient |
| Oil cognac 0.05 g.     | Water—quant. sufficient         |
| Amyl butyrate—0.075 g. | Total 2000 cc.                  |

Samples were submitted to S. Reznick, New York City, and to W. O. Winkler and the associate referee. All the residues obtained were found by Keenan to consist of hydrazino-gamma-undecalactone.

#### VOLATILE ESTERS

In the proposed method for volatile esters the distillation is accomplished with steam. This procedure has been in use in the Food and Drug Administration for many years in the determination of esters in flavors and non-alcoholic beverages. Two samples were made up to contain commercial amyl butyrate and glacial acetic acid in 50 per cent alcohol solution. The associate referee determined esters by the official method for distilled liquors, *Methods of Analysis*, A.O.A.C., 1930, p. 144, 65, and by the proposed method. Additional determinations by the proposed method were made by Winkler. The results are shown in Table 3.

TABLE 3.—*Esters in cordials, as ethyl acetate*

|         | SAMPLE A              |  | SAMPLE B              |
|---------|-----------------------|--|-----------------------|
|         | <i>g. per 100 cc.</i> |  | <i>g. per 100 cc.</i> |
| Wilson  | 0.081                 |  | 0.020                 |
|         | 0.079                 |  | 0.019                 |
|         |                       |  | } official method     |
| Wilson  | 0.078                 |  | 0.020                 |
|         | 0.079                 |  | 0.023                 |
|         |                       |  | } proposed method     |
| Winkler | 0.079                 |  | 0.020                 |
|         | 0.076                 |  | 0.018                 |

These data (Table 3) show that results by the proposed method agree very closely with those obtained by the official method for the determination of esters in distilled liquors.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended that the following methods submitted for the analysis of cordials and liqueurs be made tentative: 1. Physical Examination, 2. Specific Gravity, 3. Alcohol, 4. Total Solids, 5. Glycerol, 6.

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 57 (1936).

Sucrose, 7. Total acidity, 8. Characteristic Acids, 9. Tartaric Acid, 10. Citric Acid, 11. Malic Acid, 12. Volatile Esters, 13. Gamma-undecalactone (qualitative), 14. Optical Properties of Hydrazino- $\gamma$ -Undecalactone, 15. Methyl Alcohol, 16. Ash, 17. Soluble and Insoluble Ash, 18. Alkalinity of Soluble Ash, 19. Alkalinity of Insoluble Ash, 20. Phosphoric Acid, 21. Benzaldehyde, 22. Caramel, 23. Coal Tar Colors, 24. Aldehydes, 25. Furfural, 26. Fusel Oil.

These methods were published in *This Journal* 19, 78 (1936).

It is further recommended that during the coming year the associate referee make a search of the literature for the purpose of locating methods for the determination and detection of other constituents of cordials and liqueurs, with a view to making the section on this subject as comprehensive as possible.

The paper presented by J. B. Wilson, entitled "Note on the Determination of Thujone in Absinthe-type Liqueurs," was published in *This Journal*, 19, 120 (1936).

## REPORT ON WHISKEY, RUM, AND BRANDY

By G. F. BEYER (U. S. Bureau of Internal Revenue,  
Washington, D. C.), *Associate Referee*

The methods of analysis for whiskey, rum, and brandy outlined by the Committee for the 1935 revision of *Methods of Analysis* are, with a few minor additions and revisions, satisfactory to the Laboratory Division of the Bureau of Internal Revenue.

The specific gravity should be determined as apparent specific gravity because all distilled spirits are taxed on their apparent proof provided they contain no solid matter other than that derived from the package in which they were aged. The Marsh and paraldehyde tests are not specific for caramel coloring matter, as the coloring matter obtained from plain or untreated white oak chips gives a colored lower layer when the Marsh test is applied and a distinct turbidity is produced with paraldehyde. From the experience of this Laboratory, the paraldehyde test is absolutely useless for the purpose of confirming the presence of caramel for the reason that the coloring matter extracted from untreated white oak chips by distilled spirits gives a better test for caramel than caramel itself. Therefore, the zinc acetate test developed by Valaer and Mallory<sup>1</sup> should always be used when the Marsh test gives a positive indication on straight whiskey, rum, or brandy. In view of these facts, it is recommended<sup>2</sup> that

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 6, 475 (1934).

<sup>2</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 57 (1936).



the paraldehyde test for caramel be stricken from the official methods and the zinc acetate method be substituted therefor.

There has been some objection to the method for determining aldehydes, and it has been stated that with it it is practically impossible to obtain check results. It has been the experience of this Laboratory that when the method is closely followed and the readings made in 100 cc. Nessler tubes, which should be stoppered immediately upon the addition of the fuchsin solution, no difficulty is experienced in obtaining check results. A few notes, however, regarding this determination will not be amiss. The alcohol used in this Laboratory does not show any color with the fuchsin solution at the end of 30 minutes. Therefore, the preparation of aldehyde-free alcohol is unnecessary if the grade of alcohol purchased is Cologne Spirits, U.S.P., or analytical reagent grade having a permanganate time test of about 45 minutes, as described by Allen.<sup>1</sup> Although alcohol that complies only with the U.S.P. requirements would not be satisfactory, Cologne Spirits, U.S.P., represents about as good a grade of alcohol as can be made. Experiments have also shown that the strong aldehyde solution does lose its strength, and therefore should not be kept too long. If it should become necessary to dilute the sample before analysis, 50 per cent alcohol should be used, not water.

The fusel oil method is not entirely satisfactory as a quantitative chemical method, but if the variable composition of fusel oil in potable spirits is taken into consideration, and also the fact that the results obtained are principally used on a relative basis, the method serves its purpose quite admirably. The writer has made this determination hundreds of times and has seldom had any difficulty in obtaining check results. One suggested change in the apparatus used is recommended, that of the use of universal standard tapered ground-glass joint connections on the reflux condensers and flasks where the oxidation takes place, and the same kind of connections for the distillation of the valeric acid. These connections prevent any possible losses due to leaky corks or any soakage, as the corks become almost saturated with valeric acid. Another advantage is that the reflux condensers can be used for the refluxing of any liquid without the bother of continually changing corks.

The methods for the detection and quantitative determination of methyl alcohol outlined in *Methods of Analysis* are not satisfactory because the qualitative tests are too highly involved, and the table, 76, p. 147, given in connection with the immersion refractometer method for the quantitative estimation, is incorrect. A new table has been submitted to the referee. Experience in this Laboratory has shown that the procedure outlined by Georgia and Morales,<sup>2</sup> is as good, if not better, than any method so far suggested for the detection of methyl alcohol in spirits.

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<sup>1</sup> Commercial Organic Analysis, 5th ed. (1923).

<sup>2</sup> Ind. Eng. Chem., 18, 304 (1926).

This method is not only simple of manipulation, but it is capable of detecting very small amounts of methanol quantitatively by the use of known standards and gives a good indication of the proportion of methyl alcohol present.

It is recommended that the qualitative mercuric sulfate test for denaturants in spirits be adopted as tentative.

A paper, entitled "A Comprehensive Whiskey Analysis," was presented by Bertha Schwartz, New York City. This paper was published in the *American Wine and Liquor Journal*, Vol. 3, No. 7.

## REPORT ON FUSEL OIL

By PETER VALAER (U. S. Bureau of Industrial Alcohol,  
Washington, D. C.), *Associate Referee*

The determination of fusel oil in distilled spirits is more involved and lengthy than any made in ordinary spirit analysis, and it may be considered one of the most important.

Only a few chemical principles have been involved since the earliest recorded development of this determination. All of the methods fall into three groups: Estimation by means of a color produced by the action of sulfuric acid; extraction of the fusel oil from spirits by means of solvents, and its ultimate conversion into acids (the present method of the A.O.A.C. falls into this group); and third the extraction of fusel oil by solvents, the quantity of fusel oil being deduced from the increased volume of the solvent (Roese's method, which was the original official fusel oil method). This method lasted through only one edition of the A.O.A.C. *Methods of Analysis*, giving way to a determination of the Allen-Marquardt type.

In the present A.O.A.C. method the fusel oil is extracted by what was originally known as the Beckman process. The extracted fusel oil is oxidized by dichromates to acids and titrated and calculated to amyl alcohol. While this process is still used, the method of oxidation of the fusel oil has varied from time to time. Nitrites, permanganates, and peroxides have been used as oxidizers. The extraction process alone seems to be the most satisfactory. In this method 50 cc. of spirits are saponified, distilled, salted, and extracted four times with carbon tetrachloride, and the solvent is washed three times with saturated sodium chloride solution and twice with sodium sulfate solution. It is in the oxidizing and the final distilling steps of this method that errors have been suspected, and it is here that improvements may be made.

An extensive study of the history of the fusel oil determination shows that the Allen-Marquardt process is almost universally favored. In his

book, "Alcohol, Its Production and Application," Simmons makes the following statement: "The most precise method for the determination of higher alcohol and spirits is what is known as Allen-Marquardt Method." Schidrowitz, in the London Section, Society of Chemical Industry, June 2, 1902, said, "I am satisfied that the Allen-Marquardt method, with certain minor modifications, is alone capable of giving fairly reliable figures as far as whiskey is concerned." Other authorities—Allen, Hewett, Kaye, Tolman, Crampton—also seem to have been of the opinion that a method based on the Allen-Marquardt principles was the best.

Experiments have shown that it is practically impossible to obtain agreement in results with the three methods, but data over a period of years in this office show that excellent checks are obtained by the present A.O.A.C. determination, if careful attention is given to the details.

Some of the various objections to the Allen-Marquardt method are that it is lengthy and troublesome; that it is not scientifically accurate in that no determination of the separate alcohols is obtained, all of the higher alcohols being expressed in terms of amyl alcohol; that isopropyl alcohol is not taken care of, if present, because it is oxidized to acetone; that secondary isobutyl, if oxidized to ketone, is not recorded, and if oxidized beyond the ketone stage, will give two molecules of acetic acid for one molecule of secondary isobutyl alcohol, causing higher results; that it does not get more than 80–90 per cent of all the amyl or isoamyl alcohol present; and that butyl and propyl alcohols are much under estimated.

#### DEVELOPMENT OF METHOD UNDER AUSPICES OF A.O.A.C.

The only methods that have been selected by the A.O.A.C. are the Roese and the Allen-Marquardt. Bulletin 65, U. S. Department of Agriculture (1902), lists in *Provisional Analysis of Foods, A.O.A.C.*, under the subject of distilled liquors, the first fusel oil method, and it was used in connection with the Bromwell and Roese apparatus. The Allen-Marquardt method was not recorded. It first appeared in U. S. Department of Agriculture Circular 25 (1905). In this method 100 cc. of whiskey was used and saponified with 0.5 N sodium hydroxide and refluxed for one hour. No provision was made for standing overnight. The carbon tetrachloride was washed three times with a salt solution, but only once with sodium sulfate.

The Allen-Marquardt method appeared first as a provisional method along with the Roese method in the first edition of Bulletin 107, *Provisional Methods of Analysis*. In this method 100 cc. of whiskey was recommended, the material was saponified with 20 cc. of 0.5 N NaOH, and the mixture was saponified by boiling one hour with a reflux condenser. A suggestion was made that the mixture may stand overnight and be distilled directly. There was still only one washing with sodium sulfate.

In Bulletin 107, revised (1912) the Roese and Allen-Marquardt methods were retained as provisional; 100 cc. of whiskey was used, and there was but one washing with sodium sulfate. In 1920, *Official and Tentative Methods of Analysis of the A.O.A.C.* replaced Bulletin 107. In this edition the Roese method was dropped, and the Allen-Marquardt method became official for the first time. It was improved by the inclusion of a method for purifying carbon tetrachloride before its use. Directions for the elimination of aldehydes before the fusel oil determination and for the neutralization of the distillate to methyl orange were also incorporated.

In 1925 the method was changed slightly by the addition of three lines:

Conduct a blank determination upon 100 cc. of  $\text{CCl}_4$ , beginning the blank at that point of the procedure immediately after the extraction and just before the washings with  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  solutions.

The third and latest edition, 1930, changed the amount of whiskey used to 50 cc. for the first time, although this quantity had been used in this Laboratory for several years. This is diluted with 50 cc. of water before the 0.5 N sodium hydroxide is introduced. After oxidation, the following directions were added:

Add 100 cc. of  $\text{H}_2\text{O}$  and distil until only 50 cc. remains. Add 50 cc. of  $\text{H}_2\text{O}$  and again distil until 35–50 cc. is left.

It was suggested that extreme care be exercised to prevent the oxidizing mixture from burning and baking on the side of the distilling flask.

Up to this time 30 cc. of water had been added after oxidization, and the liquor had been distilled until only 20 cc. remained. Then 80 cc. of water was added and again distilled until 15 and 20 cc. remained. This was the most important change during the whole history of fusel oil determination in the A.O.A.C.

In the 1930 edition the distillate from the oxidizing mixture is not neutralized to methyl orange as recommended in the 1925 edition to take care of any mineral acid carried over mechanically. This step was considered to be unnecessary.

As a result of experimental work to determine how much of the higher alcohols added to standard samples were recoverable, 69–77 grams per 100 liters of primary normal amyl alcohol was recovered from a standard solution containing 100 grams per 100 liters, and 66–67 parts of isoamyl alcohol were recovered from a standard solution containing 100 grams per 100 liters. Not over 30 parts of iso-butyl alcohol was recovered from a standard sample containing over 100 grams per 100 liters, and not over 3 parts of normal propyl alcohol was recovered from a standard sample containing over 100 parts per 100 liters. As primary and iso-amyl are the principal ingredients of fusel oil, the present method, if improved so that practically all of the amyl alcohols could be recovered, would be very satisfactory.

An experiment was made to determine whether more volatile acid could be distilled over after the final distillation of the oxidized mixture. To the residue was added 50 cc. of water, and the distillation was continued. A titration of 0.2 cc. of 0.1 *N* NaOH was obtained; a second 50 cc. portion gave another 0.2 cc. of 0.1 *N* NaOH titration; and a third produced about 0.2 cc. more. This amounted to 8 parts of fusel oil per 100,000 and might account for some of the shortage in the fusel oil determination. An experiment was also conducted by titrating the oxidized fusel oil in carbon tetrachloride instead of distilling, after the method suggested by Tollman and Hillyer in Bureau of Chemistry Bulletin 122. This method gave higher results than did the present A.O.A.C. method, 90 parts per 100,000 as compared with 77 parts by the A.O.A.C. method. Other means of oxidation, such as the use of permanganate in alkaline solution, the process suggested by Mitchell and Smith, also in Bulletin 122, gave higher results than did the official method.

The two methods discussed are not necessarily correct, as all oxidized substances are titrated, and they may not necessarily be valeric acid. Experiments will be conducted to determine whether the present means of oxidization is complete by adding to carbon tetrachloride a definite amount of pure higher alcohols and determining the state of oxidization at the end of 8 hours. Various oxidizing mixtures besides the one prescribed in the A.O.A.C. method will be used.

Experiments are also being outlined (1) to determine whether final distillation is complete by running blanks with oxidizing mixtures to determine under what circumstances they furnish a titration and how far they may be distilled; (2) to use a distilling head to prevent the mineral acid from coming over mechanically and to determine if such a head would interfere with the complete recovery of valeric acid; (3) to use ground glass joints for the general improvement of the method; (4) to distil various mixtures of pure valeric acid of standard strength to determine the result of distillation and under what circumstances the distillation is complete.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended that the present A.O.A.C. method for the determination of fusel oil be retained, but that it be so improved as to make it possible to obtain most of the amyl alcohols, at least. It should be retained on account of the standards based on fusel oil determined by this method and the enormous amount of data compiled on analysis of whiskey, brandy, and rum for the past 30 years.

Those willing to collaborate in experiments conducted for the purpose of improving this method or constructing a new method may notify the Secretary of the Association.

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<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 57, 82 (1936).

## REPORT ON METHYL ALCOHOL

By E. M. BAILEY (Connecticut Agricultural Experiment Station,  
New Haven, Conn.), *Associate Referee*

In a paper published recently, *This Journal*, 18, 477 (1935), Wilson cites reviews of older methods for the detection and determination of methyl alcohol in the presence of ethyl alcohol and discusses some of the more recent procedures that have been proposed for these purposes. The present edition of *Methods of Analysis* gives several procedures for the detection of methyl alcohol, some of them being adapted to approximately quantitative interpretations. For the determination of methyl alcohol in substantial amounts in presence of ethyl alcohol the immersion refractometer method is official.

Wilson points out the limitations of present procedures and describes a quantitative procedure based upon the production and volatilization of methyl iodide and its conversion to tetramethylammoniumiodide, which is isolated and weighed. His results with known mixtures of methyl and ethyl alcohols, the first named being present in relatively small amounts as compared with the latter, indicate that methyl alcohol can be determined by this method with a high degree of accuracy. His procedure and the necessary apparatus and reagents are given in detail in the paper cited.

It seemed desirable to study this method collaboratively for the purpose of including it among the official or tentative methods of this Association. Accordingly the associate referee prepared a series of samples containing varying proportions of methyl alcohol in approximately 35 per cent ethyl alcohol and submitted them to several collaborators.

The collaboration was provided through the courtesy of Wendell Vincent and W. R. M. Wharton of the U. S. Food and Drug Administration. The chemists taking part and reporting results were M. L. Yakowitz, San Francisco Station, C. A. Wood, New York Station, and W. T. Mathis and C. E. Shepard of the Connecticut Agricultural Experiment Station.

The Beverage Laboratory of the Food and Drug Administration, Washington, D.C. generously supplied the apparatus required as well as photostats of the same to enable proper interpretation of the method submitted.

The method sent out was exactly as described by the author. The samples sent out contained methyl and ethyl alcohol in such proportions and dilutions as would be within the limits cited in the method for a 20 cc. aliquot, but it was subsequently noted that the precipitate to be weighed was of such magnitude that it could not be manipulated conveniently, and directions for further dilution of two of the samples were forwarded.

The comments of collaborators indicate or suggest that considerable preliminary work was necessary before satisfactory results could be obtained. This was particularly true in the associate referee's laboratory, where considerable trouble was experienced in controlling the reaction. At first the reaction was so delayed and so incomplete that low and uncertain results were obtained. With a new lot of both iodine and phosphorus the reaction was so violent that difficulty was had in checking it. Thus one collaborator (W.T.M.), observed: "With extreme care fairly concordant results were obtained with Samples 1 and 2, but that could not be done with Sample 3." He further states: "Attempts to control the reaction were made by (1) cooling the reaction flask to  $-4^{\circ}\text{C}$ . before adding the sample, and (2) by adding 5 cc. of 50 per cent alcohol immediately before adding the sample. These modifications were not successful, but the latter furnished a hint that the alcohol present might be a factor. When 2.5 cc. of 95 per cent alcohol was added the iodine was dissolved, ready mixing of the phosphorus took place, and no violent reaction took place when the sample was added. After the sample had been added the reaction could be initiated and controlled according to the directions given in the method. This preliminary addition of alcohol appeared to solve the difficulty regardless of the dilution of the sample added. Moreover the first lots of phosphorus and iodine, with which the delayed and incomplete reaction was obtained, worked perfectly under the new conditions.

No other collaborator remarked on the control of the reaction or the initiation of it. One of them (M.L.Y.), remarked that "preliminary trials indicated that the charge of methyl alcohol should be such as to yield not more than one gram of precipitate in order to insure a considerable excess of trimethylamine in the receiving flask beyond that required to combine with the methyl iodide formed, and also to facilitate manipulation of the precipitate of tetramethylammoniumiodide. He further suggested that saturated wash solution be used in the receiving flask, instead of absolute alcohol, even when the expected precipitate amounts to as much as 1 gram. This collaborator used a 20 cc. aliquot of Sample 1, 10 cc. of Sample 2, and a 10 cc. aliquot of Sample 3 after diluting to 1+9.

Comments of another collaborator (C.A.W.) do not indicate that any substantial modification of the method as sent out was made. He remarks that a very low result was obtained on Sample 1 when an aliquot of 20 cc. was used. In all other trials 10 cc. portions were used.

Table 1 gives the results reported by the several collaborators. The figures should be interpreted in the light of the discussion based upon the comments of the analysts collaborating.

The results reported by collaborator C.A.W. for Samples 2 and 3 are of the same order as preliminary results obtained in the associate referee's laboratory by collaborator W.T.M.

TABLE 1.—*Methyl alcohol in mixtures with ethyl alcohol (by gravimetric determination of tetramethylammoniumiodide)*

| COLLABORATOR   | METHYL ALCOHOL |       |            |       |            |       |
|----------------|----------------|-------|------------|-------|------------|-------|
|                | SAMPLE 1       |       | SAMPLE 2   |       | SAMPLE 3   |       |
|                | PRESENT        | FOUND | PRESENT    | FOUND | PRESENT    | FOUND |
|                | g./100 cc.     |       | g./100 cc. |       | g./100 cc. |       |
| M. L. Yakowitz | 0.100          | 0.091 | 1.000      | 1.06  | 10.000     | 9.96  |
|                |                | 0.096 |            | 1.07  |            | 10.02 |
|                |                | 0.096 |            | 1.04  |            |       |
|                |                |       |            |       |            |       |
| W. T. Mathis   | 0.100          | 0.114 | 1.000      | 0.994 | 10.00      | 10.01 |
|                |                | 0.107 |            | 0.989 |            | 9.79  |
|                |                | 0.112 |            |       |            | 10.01 |
|                |                |       |            |       |            |       |
| C. A. Wood     | 0.100          | 0.109 | 1.000      | 0.774 | 10.00      | 9.04  |
|                |                | 0.111 |            | 0.778 |            | 8.98  |

It appears that the procedure outlined in the instructions to collaborators should be modified—

(1) To direct that the aliquot of sample used in the determination contain not more than 0.160 gram of methyl alcohol.

(2) To direct that 2.5 cc. of 95 per cent alcohol be introduced into the reaction flask immediately before the sample is introduced.

(3) To advise in all cases the use of 25 cc. of wash solution in the receiving flask instead of 25 cc. of absolute alcohol.

The method is based upon sound chemical principles, and it affords an accurate gravimetric procedure for the determination of small amounts of methyl alcohol in the presence of relatively large amounts of ethyl alcohol. From the experience of collaborators, however, it seems advisable to revise the description in the three respects herein noted. It is thought that these modifications will make the procedure more workable in the hands of analysts using it for the first time and avoid the necessity of so much preliminary trial. Although some of the results reported may be regarded as perfect, others have not the accuracy expected of a precision method. Whether the revision suggested will result in more uniform behavior and more concordant results cannot be determined without trial, and it is recommended<sup>1</sup> that the method be revised as suggested and resubmitted to collaborative study.

As a supplement to this work it seemed desirable to retest the method for determining methyl alcohol that now appears in *Methods of Analysis*, 1930, p. 476. This is a tentative method, and it gives results that are closely approximate to the truth in the experience of those who have used it. Some doubt has been raised as to the value of this and similar

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 77 (1936).



procedures, and accordingly collaborators were asked to try this method on at least two (Nos. 1 and 2) of the collaborative samples sent out for study of the tetramethylammoniumiodide method. Two of them submitted the results reported in Table 2.

TABLE 2.—*Methyl alcohol by procedure in Methods of Analysis, p. 476*

| COLLABORATOR | METHYL ALCOHOL, g./100 cc. |              |                |              |                |              |
|--------------|----------------------------|--------------|----------------|--------------|----------------|--------------|
|              | SAMPLE 1                   |              | SAMPLE 2       |              | SAMPLE 3       |              |
|              | <i>present</i>             | <i>found</i> | <i>present</i> | <i>found</i> | <i>present</i> | <i>found</i> |
| C.A.W.       | 0.10                       | 0.10         | 1.00           | 0.82         | 10.0           | —            |
| C.E.S.       | 0.10                       | 0.09         | 1.00           | 1.00         | 10.0           | 9.9          |

Earlier results that formed the basis for the adoption of this method are found in *This Journal*, 11, 324 (1928) and 12, 262-3 (1929). The amounts of methyl alcohol involved were of magnitudes of 1 and 2.5 per cent, and from the above results (Table 2) it appears that the method gives satisfactory approximations for amounts from 0.1 to 10.0 gram per 100 cc. The advantages of the procedure are that it affords a quantitative approximation in conjunction with an official (U.S.P.) qualitative test; no special apparatus is required; and the manipulation is very simple. Its usefulness for practical purposes at least, seems to be demonstrated, and its retention as a tentative method is warranted.

## REPORT ON STANDARD SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

In the 1930 edition of *Methods of Analysis*, in the chapter on fertilizers, are found procedures for making standard solutions of hydrochloric acid, sulfuric acid, and sodium hydroxide, but instructions are not given in detail. These methods specify standardization of hydrochloric acid by precipitation of silver chloride, of sulfuric acid by the precipitation of barium sulfate, and of sodium hydroxide with Bureau of Standards benzoic acid. Hydrochloric acid and sulfuric acid solutions also may be standardized by titration with standard sodium hydroxide that has been standardized with benzoic acid.

Throughout this publication, with a few notable exceptions where special standard acid and alkali are needed, no further methods are given for the preparation of these solutions. Instructions usually specify titration with standard solutions of varying designated strengths and refer to the fertilizer chapter.

Since the current methods were developed, the Bureau of Standards has prepared potassium acid phthalate as a primary standard. This com-

pound is soluble in water, and therefore its use does not involve the disadvantage of the use of the alcoholic solvent.

In this report the referee presents the results of a study of the preparation of a carbon dioxide-free alkali solution, and the relative values of standard acid as standardized by the precipitation of silver chloride and against standard alkali by titration; and also the details of methods for the preparation and standardization of sodium hydroxide and hydrochloric acid.

#### PREPARATION OF ALKALI STOCK SOLUTION

There are numerous methods of preparing standard sodium hydroxide solutions which contain very little or no sodium carbonate, such as the preparation from sodium metal,<sup>1</sup> precipitating the carbonate with alkaline earth ions,<sup>2</sup> or precipitating the carbonate in concentrated alkali solution.<sup>3</sup> If the standard alkali solution is to be free from carbonate, a barium hydroxide solution which has been treated with a barium salt<sup>4</sup> is convenient.

Considering the general methods indicated above, the referee decided that it would be advantageous to confine the work at this time to a study of concentrated sodium hydroxide as a source of carbonate-free alkali. Solutions were prepared containing a 1+1 mixture of sodium hydroxide and a saturated solution of sodium hydroxide. Carbon dioxide was determined in both.

Carbon dioxide as determined by the evolution method<sup>5</sup> in two different concentrations of sodium hydroxide, with or without added carbonate, and in the reagent sodium hydroxide, which was used as a source of material, is given in Table 1.

TABLE 1.—Carbon dioxide as determined by evolution method

| MATERIAL   | SODIUM CARBONATE IN TOTAL ALKALI |          |
|--|----------------------------------|----------|
|  | per cent                         | per cent |
| 1. Reagent NaOH                                  | 1.01                             | 0.95     |
| 2. Saturated NaOH                                | 0.14                             | 0.14     |
| 3. 1+1 NaOH soln (a) filtered                    | 0.14                             |          |
| (b) siphoned                                     | 0.14                             |          |
| 4. 10 g. $\text{Na}_2\text{CO}_3$ } (c) filtered | 0.16                             |          |
| 190 g. NaOH } (d) siphoned                       | 0.14                             |          |
| 200 cc. $\text{H}_2\text{O}$ }                   |                                  |          |
| 5. 25 g. $\text{Na}_2\text{CO}_3$ }              | 0.53                             |          |
| 75 g. NaOH }                                     |                                  |          |
| 100 cc. $\text{H}_2\text{O}$ }                   |                                  |          |

<sup>1</sup> F. W. Kuster, *Z. anorg. Chem.*, **13**, 134 (1897); **41**, 474 (1904); Cornog, *J. Am. Chem. Soc.*, **43**, 2573-4 (1921); Stahl, *Z. anal. Chem.*, **97**, 86-9 (1934).

<sup>2</sup> E. Newberry, *Ind. Chemist*, **3**, 462 (1927); Clark, *The Determination of Hydrogen Ions*, p. 197. Williams & Wilkins (1928); Kolthoff & Furman, *Volumetric Analysis*, Vol. II, p. 77. Wiley (1929); Han & Chao, *Ind. Eng. Chem.*, **14**, 229 (1932).

<sup>3</sup> *J. Am. Chem. Soc.*, **30**, 1192 (1908); Sorenson, *Biochem. Z.*, **21**, 186 (1909).

<sup>4</sup> Kolthoff & Furman, *Volumetric Analysis*, Vol. II, p. 75. Wiley (1929).

<sup>5</sup> *Methods of Analysis*, A.O.A.C., 1930, 115.

The saturated solution of sodium hydroxide was unsatisfactory because a longer time was required to obtain a clear supernatant liquid than in the case of the 1+1 solution, and the concentration varied from day to day due to the precipitation during the cool nights of crystals of sodium hydroxide. Since the solubility of sodium carbonate was repressed in the 1+1 solution, to the same degree as in the saturated alkali, the former was used as the stock reagent for standard solutions of alkali. Table 1 shows that the carbonate content of the reagent sodium hydroxide up to 6 per cent does not affect the amount of sodium carbonate in the clear liquid. With a contamination of about 25 per cent, however, there was a marked increase in the amount of carbonate in the 1+1 solution.

Han and Chao<sup>1</sup> determined that the carbonate content of 1+1 sodium hydroxide, after dilution with CO<sub>2</sub>-free water to 1.0 *N*, was 0.0048–0.0095 gram of actual Na<sub>2</sub>CO<sub>3</sub> per 100 cc., or 0.09–0.18 per cent sodium carbonate in total alkali. Their detailed procedure for the volumetric estimation of Na<sub>2</sub>CO<sub>3</sub> in sodium hydroxide is rapid and accurate and may be applied to 0.1 *N* solutions by using a 100 cc. sample instead of 45 cc. of normal NaOH.

#### PREPARATION OF CO<sub>2</sub>-FREE WATER

The preparation of CO<sub>2</sub>-free distilled water was accomplished by boiling distilled water for 30 minutes in a Pyrex flask or by passing CO<sub>2</sub>-free air through water for 12 hours. On account of the exceedingly small amounts of carbon dioxide expected, it was not practicable to apply the absorption method. Carbon dioxide was accordingly determined by titrating 100 cc. of water with 0.01 *N* alkali and using phenolphthalein as an indicator. After boiling for 30 minutes distilled water showed a titration equivalent to 0.12 cc. of 0.01 *N* alkali. The water treated by bubbling CO<sub>2</sub>-free air through it for 12 hours showed a titration of 0.13 cc. of 0.01 *N* alkali.

These titration values for distilled water are nearly identical with the indicator correction described by Kolthoff and Furman.<sup>2</sup> It is concluded, therefore, that distilled water freed of carbon dioxide by either method will contain no significant amounts.

Using 1+1 alkali and preparing a 0.1 *N* solution by diluting with boiled water, the referee found on two experiments carbon dioxide equivalent to 0.86 mg. and 0.74 mg. of sodium carbonate per 100 cc. Analysis of 0.1 *N* alkali solution prepared from 1+1 alkali solution filtered through a glass crucible was identical with the above.

The literature cited indicates the extreme difficulty of preparing an alkali solution absolutely free from contamination with sodium carbonate. A standard solution prepared from the 1+1 sodium hydroxide mixture with CO<sub>2</sub>-free water will show a maximum variation from a solution

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Volumetric Analysis*, Vol. II, p. 69. Wiley (1929).

absolutely free from carbon dioxide of not more than 0.2 cc. in a titration of 100 cc. The maximum contamination reported above is 0.86 mg. of  $\text{Na}_2\text{CO}_3$  per 100 cc. of 0.1 *N* alkali, and in this statement allowance is made for the possibility of contamination greater than that reported. If the standard alkali solution is used with the same indicator as that with which it was standardized, there will be no error due to the small amount of carbon dioxide indicated previously. Therefore, the referee concludes that for all except extraordinary cases the very small amount of carbonate contamination occasioned by the use of the 1+1 alkali stock solution is not significant. In any event, this small amount of carbonate is to be preferred to such substances as barium or calcium, which would be required to effect its removal. In the few cases where absolute freedom from carbon dioxide is required, it will be necessary to devise special solutions of barium hydroxide or some similar substance. This is work which can well be deferred for the future.

The method for preparing standard solutions of sodium hydroxide has been published, *This Journal*, 19, 107 (1936).

#### HYDROCHLORIC ACID

Numerous procedures have been advocated to determine the active strength of hydrochloric acid solutions. Direct methods of measurement include such primary standards as calcite,<sup>1</sup> sodium oxalate,<sup>2</sup> borax,<sup>3</sup> sodium carbonate,<sup>4</sup> and mercuric oxide.<sup>5</sup> Of these only sodium oxalate is prepared by the U. S. Bureau of Standards,<sup>6</sup> and since the Bureau<sup>7</sup> does not certify it as an acidimetric standard, sodium oxalate and other primary standards quoted above, whose purity must be determined in the laboratory, have not been considered at this time.

The only objection to such a standard as mercuric oxide is that it contains impurities of the order of one part per thousand.<sup>8</sup> Therefore, considerable work would be necessary in preparing and analyzing a sample for use as a primary standard.

Many analytical texts direct the standardization of hydrochloric acid solutions by precipitating and weighing as silver chloride; and this method is one used by the Association of Official Agricultural Chemists.<sup>9</sup> Some work was done by this procedure. Likewise the method involving titration with standard sodium hydroxide was studied.

The method for preparing standard solutions of hydrochloric acid has been published, *This Journal*, 19, 108 (1936).

<sup>1</sup> Hammer, *J. Chem. Ed.*, 11, 245-7 (1934).

<sup>2</sup> Sorenson, *Z. Anal. Chem.*, 36, 639 (1897); 42, 333, 512 (1903); Bur Standards Cir 381, p. 9.

<sup>3</sup> Kolthoff and Furman, *Volumetric Analyses*, Vol. II, pp. 93-96. Wiley (1929).

<sup>4</sup> G. Lunge, *Z. angew. Chem.*, 10, 522 (1897); 17, 231 (1904); 18, 1520 (1905).

<sup>5</sup> Ince, *Z. anal. Chem.*, 56, 177 (1917); 57, 176 (1918).

<sup>6</sup> See Standard Samples Issued or in Preparation by the National Bureau of Standards, p. 10, Feb. 18, 1935.

<sup>7</sup> Bur. Standards Cir. 381, p. 9.

<sup>8</sup> Kolthoff and Furman, *Volumetric Analysis*, Vol. II, pp. 93-101, Wiley (1929).

<sup>9</sup> *Methods of Analysis*, A.O.A.C., 1930, 18-19.

It had been hoped to present a gravimetric or a direct method of standardization which would yield a normality value accurate to one part in a thousand. The method involving precipitation with silver nitrate was tried on a solution of 0.1 *N* hydrochloric acid to compare it with the normality value determined by titration with standard alkali prepared as above. Six determinations gave values for the standardization against sodium hydroxide, using phenolphthalein, of 0.10084, 0.10088, 0.10084, 0.10084, 0.10084, and 0.10084. Five determinations standardized gravimetrically gave 0.10074, 0.10063, 0.10065, 0.10066, and 0.10062. The difference in results is due mostly to the solubility of silver chloride as shown by Morey,<sup>1</sup> who found from 0.1 to 0.3 mg. of hydrochloric acid in the filtrate and washings of four precipitations of the chlorides in a 0.1 *N* hydrochloric acid solution. The error, therefore, in the above gravimetric method due to the solubility of the chlorides in the filtrate and washings would be from 0.6 to 1.8 parts per thousand.

#### INDICATORS

In acidimetry and alkalimetry there are available many indicators in which, when correctly used, the indicator error is negligible. For the determination of strong bases and strong acids, the indicator change should occur at a *pH* between 4 and 10.<sup>2</sup> If the solutions are weaker, as 0.01 *N*, the indicator color change should be closer to *pH*-7. When a strong base is used to titrate a weak acid, as acetic, the equivalence point is *pH*-8.87,<sup>3</sup> and indicators whose color changes at a *pH* between 7.75 and 10 are necessary.

The *pH* range for a number of indicators in general use follows:

*Table of indicators<sup>4</sup>*

| INDICATORS               | SYNONYM                         | <i>pH</i> RANGE |
|--------------------------|---------------------------------|-----------------|
| Thymol                   | Thymolsulfonphthalein           | 1.2- 2.8        |
|                          |                                 | 8.0- 9.6        |
| Methyl Orange            | Tropeolin D                     | 2.9- 4.0        |
| Bromophenol Blue         | Tetrabromophenolsulfonphthalein | 3.0- 3.6        |
| Sodium Alizarinsulfonate | —                               | 4-5             |
|                          |                                 | 5-6             |
| Methyl Red               | —                               | 4.2- 6.3        |
| Bromocresol Purple       | Dibromo-o-cresolsulfonphthalein | 5.2- 6.8        |
| Bromothymol Blue         | Dibromothymolsulfonphthalein    | 6.0- 7.6        |
| Phenol Red               | Phenolsulfonphthalein           | 6.8- 8.4        |
| Thymol Blue              | (see above)                     | 8.0- 9.6        |
|                          |                                 | 1.2- 2.8        |
| Phenolphthalein          | —                               | 8.3-10          |
| Thymolphthalein          | —                               | 9.3-10.5        |

<sup>1</sup> *J. Am. Chem. Soc.*, 34, 1029 (1912).

<sup>2</sup> Hillebrand and Lundell, *Applied Inorganic Analysis*, p. 129. Wiley (1929); Kolthoff and Furman, *Volumetric Analysis*, Vol. I, p. 98. Wiley (1928).

<sup>3</sup> *Ibid.*, Vol. II, p. 101.

<sup>4</sup> Taken from Handbook of Chemistry and Physics, pp. 573-4, Chemical Rubber Pub. Co., 7th ed.

RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the methods submitted by the referee for the preparation and standardization of acid and alkali be adopted as tentative methods.

(2) That the method for standardization of alkali solution, *Methods of Analysis*, A.O.A.C., 1930, 19, (c), beginning "Accurately determine the" and continuing to the end of the paragraph, be deleted.

(3) That the referee attempt to devise a direct method for standardizing acids.

(4) That methods be studied for the preparation of standard solutions other than those used in acidimetry and alkalimetry.

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 46 (1936).

## MONDAY—AFTERNOON SESSION

### REPORT ON EGGS AND EGG PRODUCTS

By H. A. LEPPER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The report by the Associate Referee on Detection of Decomposition was the only one made on the work contemplated for this year. His recommendations are approved by the referee. From studies on unsaponifiable constituents a report of progress is made on the development of methods for the determination of cholesterol.

A tentative method for the determination of unsaponifiable matter is given in the chapter on Eggs and Egg Products. The procedure follows the details of the F. A. C. method given in the Chapter on Oils and Fats. The Referee on Cereals this year is recommending that the F. A. C. method, as adapted for flour and macaroni products, be deleted from *Methods of Analysis, A.O.A.C.* and that the Kerr-Sorber modified method be retained for unsaponifiable matter in these products. In the development of methods for cereal products, noodles, and eggs, uniformity of procedure has been retained wherever possible, especially <sup>of the</sup> methods the chief purpose of which is to furnish a basis of interpretation of egg content. The present method for unsaponifiable matter in eggs was adopted after a one-year study, *This Journal*, 12, 347 (1929). A few years later, *Ibid.*, 14, 398 (1931), the Referee on Eggs, in considering the advisability of further study of the method for official adoption, stated: "The value of this method at the present time is not sufficient to justify more work on the present method." The constituents of the unsaponifiable matter appear to offer more promise in estimating egg content than does the total amount. Studies are progressing on this determination. No useful purpose is evident in retaining the present tentative method in the forthcoming revision of *Methods of Analysis*. Although Hertwig, *This Journal*, 9, 122 (1926), proposed an adaptation of the Kerr-Sorber method for eggs, no studies of the method followed his proposal. It is not believed that adequate directions are available for its adoption in place of the present method.

The methods for dextrose and sucrose have been adopted as official, first action. Although the results of collaborative study, *This Journal*, 16, 298 (1933), are satisfactory, the need for correction factors for the effect of the volume of insoluble matter should be determined before the methods are finally adopted as official.

Further study of the other tentative methods in the chapter on eggs and of those adopted since the last revision of the book is necessary before their status can be changed.

RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That the title of sec. 14, Chapter XXIII, p. 248, be changed from "Acidity of Fat" to "Acidity of Ether Extract."
- (2) That the rapid method for the determination of acidity of ether extract, *This Journal*, 15, 341 (1932), be further studied.
- (3) That the method for ammonia nitrogen be adopted as tentative for liquid eggs and study continued.
- (4) That studies of methods for the determination of acid-soluble phosphoric acid be continued.
- (5) That study of the method for water-soluble and crude albumin nitrogen in dried eggs be continued.
- (6) That methods for the determination of sugars, added salt, and qualitative and quantitative glycerol be further studied.
- (7) That methods for the determination of constituents of the unsaponifiable matter and the determination of fat by acid hydrolysis be continued.

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No report on unsaponifiable constituents and fat was given by the associate referee.

## REPORT ON DETECTION OF DECOMPOSITION IN EGGS

By JOSEPH CALLAWAY, JR. (U. S. Food and Drug Administration, New York), *Associate Referee*

A Referee on Eggs and Egg Products was first appointed by the Association in 1918, and some of the first work reported was on the detection of decomposition in shell, liquid, and frozen eggs. Later Referee H. L. Lourie recommended that the methods of analysis reported in the U. S. Dept. Agr. Bull. 846 for the detection of decomposition be adopted by the Association as tentative, *This Journal*, 6, 13 (1922). The Committee on Recommendations of Referees did not approve Lourie's recommendation, however, and recommended that collaborative work be done in the Association before these methods were adopted. This project then lapsed for several years while work was being done on the composition of eggs and on methods for determining the amount of egg substance in noodles, etc. In 1923, H. I. Macomber was appointed as Associate Referee on Decomposition in Eggs. He studied the determination of the acidity of the ether extract in liquid and dried eggs and later the acid-soluble phosphoric acid. A method for determining the acidity of the ether extract was adopted as official under the title "acidity of the fat." As it is evident

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 58, 93 (1936).



that the material on which the acidity is determined is crude ether extract and that it contains phospholipoids, which may have an acid reaction, the title should be changed to "Acidity of Ether Extract."

Following Macomber, Associate Referee H. D. Grigsby studied a rapid method for the determination of the acidity of the ether extract in dried eggs.

As a result of the work done in various laboratories of the U. S. Department of Agriculture on means of measuring decomposition in eggs the following points seem to be well established: (1) Freshly laid eggs of healthy fowl are for practical purposes free from bacteria, (2) spoilage of eggs in the shell is due to the development of the embryo or to the invasion of bacteria, which grow and develop enzymes, resulting in decomposition of the constituents of the egg. Owing to the varying types of bacteria that utilize the different components of the egg for food and to the various enzymes produced, which act on different constituents of the eggs, one or more of the following types of decomposition may occur:

Protein, believed to be associated with formation of loosely bound ammonia and of indol and skatol.

Carbohydrate, associated with a decrease of copper-reducing substances in liquid eggs.

Fat, associated with an increase of the acidity of the ether extract.

Lecithin, associated with increase in acid-soluble phosphoric acid, and possibly with increase of acidity of ether extract.

When eggs are removed from the shell the types of decomposition described above will take place in the liquid egg unless methods are used to prevent bacterial growth. In this country freezing the liquid egg and holding at low temperatures, and drying are the two important methods used. If dried sufficiently, eggs may be kept at ordinary temperatures, but they must be refrigerated if more than a small amount of moisture is present.

Work on dried eggs has shown that ammonia nitrogen is lost in the drying process and the copper-reducing substances are so changed as to make that determination unreliable, but that the acidity of the ether extract of the dried egg bears a direct relationship to the acidity of the ether extract in the liquid eggs. Therefore this determination is a good indication of whether or not the dried eggs were produced from sound liquid eggs. However, it should be remembered that it is possible to have decomposition in eggs without a breaking up of those components that give rise to a high acidity of the ether extract. Acid-soluble phosphoric acid also passes with little change from liquid to dried eggs, so this also is an indication of the condition of the liquid eggs before being dried, insofar as decomposition of lecithin and related substances are concerned. The associate referee knows of no reported work on detection of decomposition occurring in dried eggs after manufacture.

Many shell eggs are broken out and separated into yolks and whites for either freezing or drying. Frozen yolk can be examined by the methods used for whole eggs, and dried yolk by the method used for dried whole eggs, but the Association has no method for the detection of decomposition in frozen or dried egg white.

It is evident that much additional work is necessary before the chapter on eggs in *Methods of Analysis, A.O.A.C.* contains adequate methods for the detection of decomposition.

Last year Subcommittee C on Recommendation of Referees recommended that the study of these methods be continued, with attention given (1) to ammonia nitrogen as an index of decomposition; (2) to the applicability of the rapid method for the acidity of the fat to both liquid and dried eggs; and (3) to the determination of acid-soluble phosphoric acid. The associate referee decided that the determination of ammonia nitrogen was most important.

A method for this determination was also outlined in Bulletin 846, U. S. Department of Agriculture, and in *This Journal*, 6, 7 (1922). Although for collaborative study it is necessary that the analysts participating have the same egg material, since it is difficult to prevent decomposition of mixed eggs by preservatives and since the freezing and holding frozen of small egg samples introduce many problems requiring further study, it was decided to secure collaborative results by having analysts in the same laboratory determine ammonia nitrogen on samples from the same lot of mixed eggs, the samples to be taken at practically the same time. The associate referee secured the shell eggs and prepared the mixed lots. Samples were withdrawn by him and then turned over to another analyst, who withdrew samples within an hour of the taking of the first samples. The determinations were made with different apparatus and the titrations with different solutions. The method used was that cited previously. The results are given in Table 1.

TABLE 1.—*Ammonia nitrogen*  
(mg. per 100 grams of sample)

|                             | SAMPLE 1 * | SAMPLE 2 | SAMPLE 3 | SAMPLE 4 |
|-----------------------------|------------|----------|----------|----------|
| Collaborator                |            |          |          |          |
| Associate referee           | 1.9        | 2.2      | 5.6      | 9.3      |
|                             | 1.9        | 2.2      | 5.4      | 9.5      |
| W. E. Kirby, New York Sta.  | 1.9        |          |          | 9.8      |
|                             |            |          |          | 10.4     |
| B. B. Wright, New York Sta. | 1.9        | 2.1      | 6.1      |          |
|                             |            | 2.0      | 6.4      |          |

\* Sample 1, fresh eggs; 2, storage eggs; 3, part of Sample 2, which stood overnight at room temperature, mixed with fresh eggs; 4, part of Sample 3 held 4 days in ice box then mixed with some fresh mixed eggs.

It will be seen that reasonably concordant results were obtained. A consideration of the method used indicates that the amount of air that

must necessarily be used to carry over the ammonia is only roughly regulated. Actually, with the apparatus described the amount of air is to a certain extent regulated if the operator blows the egg solution as much as possible without making it splash into the trap. In order to secure a better control on the amount of air used, the associate referee proceeded as follows:

A solution of ammonium chloride was prepared which contained 5 per cent more ammonium chloride than a 0.02 *N* solution; 20 cc. of this solution instead of an egg sample was introduced into an evolution tube. The ammonia evolved was passed through 75 cc. of water, to which had been added 20 cc. of 0.02 *N* sulfuric acid and methyl red indicator solution to give a deep red color to the solution. When enough ammonia had been carried over to neutralize the 20 cc. of acid, as shown by a change of color of the indicator, it was assumed that enough air would be used to remove any amounts of ammonia which might normally occur in even badly decomposed eggs. In practice the blowing was continued for 30 minutes after the indicator changed color. With the apparatus used by the associate referee, about 3.5 hours were required to evolve the ammonia from the 20 cc. of special ammonium chloride solution. It is believed that this means of controlling the time of blowing is preferable to that recommended by Lourie. Since a certain amount of the alkalized solution will be carried over mechanically, a blank for each cylinder should be obtained by making a determination with all reagents, but with no egg samples. The blowing should be at the same rate and for the same length of time as that measured by the control cylinder containing 20 cc. of the special ammonium chloride solution.

The associate referee recommends that the method submitted be adopted as tentative for liquid eggs. The method is essentially as published previously in *This Journal*, 6, 7 (1922).

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That work on the method for ammonia nitrogen be continued in order that this method may be made official.
- (2) That a study be made of the present method for acidity of ether extract to determine whether the acidity measured is in part due to a titration of lecithin or related compounds and not free fatty acid.
- (3) That a study be made of reported methods for the determination of acid-soluble phosphoric acid, and of data on eggs secured by these methods. If it appears desirable to have a method for acid-soluble phosphoric acid adopted by the Association, it is recommended that collaborative work be started on a method chosen by the associate referee.

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 58 (1936).

No report on glycerol, sugar, and added salt was given by the associate referee.

No report on dried eggs was given by the associate referee.

## REPORT ON PRESERVATIVES

By JOHN C. KRANTZ, Jr. (State of Maryland Department of Health, Baltimore, Md.), *Referee*

One of the most striking studies conducted last year in the field of food preservatives consisted of a comprehensive investigation of the relative efficacy of preservatives by Jensen and Orner.<sup>1</sup> The organisms employed were: *Saccharomyces Pastorianus*, *S. apiculatus*, *Torula alba*, *T. rubra*, *Aspergillus glaucus*, *A. flavus*, *A. niger*, *A. fumigatus*, *Penicillium glaucum*, *Citromyces pfefferianus*, *Cladosporium herbarum*, *Dematium pululans*, *Mucor racemosus*, *M. Mucedo*, and *Rhizopus nigricans*. These were the strongest strains. It was observed that, of the organisms collected from the air in July, 75 per cent were moulds and only 25 per cent bacteria, the majority of the latter being saprophytic. Forms of *Penicillium* and *Citromyces* are the most common, followed by *Aspergillus* and sometimes *Mucor*. A petri dish of glucoseagar exposed to the air for 48 hours, was found to give colonics as follows:— *Verticillium* 1, micrococci 2, fluorescing bacteria 8, *Cladosporium* 9, *Citromyces* 11, yeasts 11, *Rhizopus* 12, *Aspergillus* 14, *Mucor* 18, *B. subtilis* 21, *Penicillium* 37, *Torula* 71. As a summary of a large number of detailed results, the following figures show the greatest dilution at which the growth of all the organisms was prevented.

|                                 | GREATEST<br>EFFECTIVE<br>DILUTION | PREVIOUSLY<br>DETERMINED DILUTIONS<br>FOR BACTERIA |
|---------------------------------|-----------------------------------|--|
| Sodium benzoate                 | 50                                | 100  |
| Methyl p-hydroxybenzoate        | 200                               | 450  |
| Euquinine                       | 300                               | 150  |
| Ethyl p-hydroxybenzoate-sodium  | 400                               |  |
| Propyl p-hydroxybenzoate-sodium | 500                               | 500  |
| Methyl p-hydroxybenzoate        | 666                               | 800  |
| Propyl p-hydroxybenzoate        | 700                               | 800  |
| Benzoic acid                    | 900                               | 100  |
| Trypaflavin                     | 1500                              | 3000   |
| Benzyl p-hydroxybenzoate-sodium | 1700                              | 1000   |
| Methylene blue                  | 2000                              | 5000   |
| Hexylresorcinol                 | 2500                              | 1500   |
| Rivanol                         | 2500                              | 2500   |
| Chinosol                        | 3000                              | 2000   |
| Methyl violet                   | 3500                              | 9000   |
| Brilliant green                 | 3500                              | 6000   |
| Malachite green                 | 5000                              | 3000   |

<sup>1</sup> *Dansk Tids. Farm.*, 8, 233 (1934).

The series obtained for the dilutions which killed in 48 hours was very similar. The results were affected to a great extent by the nature of the substrate.

In accordance with the suggestion of the Chairman of the Committee on Recommendations of Referees the newly adopted quantitative method for the determination of saccharin in non-alcoholic beverages was broadened in its scope to include all foods that may contain saccharin. Pursuant to this end the following changes in the language of the present method are recommended for collaborative study.

### SACCHARIN

#### PREPARATION OF SAMPLE

(a) *Non-alcoholic beverages*.—Proceed as directed under 13 or 14<sup>1</sup> without preliminary treatment.

(b) *Fruit juices and sirups*.—Transfer with a little H<sub>2</sub>O, 50 cc. of the sample to a 100 cc. volumetric flask. Dilute to about 75 cc. with H<sub>2</sub>O, add 3 cc. of glacial acetic acid, and mix. Add a slight excess of 20% neutral Pb acetate soln, mix thoroughly, dilute to the mark with H<sub>2</sub>O, and again mix thoroughly and filter. Transfer 50 cc. of this filtrate to a separatory funnel and proceed as directed under 13 or 14.

(c) *Alcoholic beverages*.—Heat 100 cc. of the liquid on a steam bath to remove alcohol (accomplished in most cases by evaporating to half the original volume). With heavy sirups, dilute the liquid with an equal volume of H<sub>2</sub>O before beginning the evaporation. After the alcohol has been removed, transfer to a separatory funnel with the aid of a little H<sub>2</sub>O and proceed as directed under 13 or 14.

(d) *Solid or semi-solid preparations*.—Using a little hot H<sub>2</sub>O transfer 50 g. of the sample to a 100 cc. volumetric flask and add sufficient boiling H<sub>2</sub>O to make the volume about 75 cc. Allow the mixture to stand about 2 hours, shaking occasionally. Then add 3 cc. of glacial acetic acid and mix thoroughly. Add a slight excess of 20% neutral Pb acetate soln, dilute to the mark with cold H<sub>2</sub>O, mix, allow to stand for 20 min., and filter. Transfer 50 cc. of the filtrate to a separatory funnel and proceed as directed under 13 and 14.

The present official qualitative test for saccharin is the organoleptic test after extraction of the food product. A more decisive method based on the work of Tortelli and Piazza<sup>2</sup> and modified by others<sup>3</sup> was studied. The method consists of extracting the material in the usual manner. The residue is further extracted with a mixture of equal volumes of absolute ethyl ether and petroleum ether. The residue obtained by the evaporation of this ethereal extract is treated with phenolsulfonic acid and thus converted into phenolsulfonphthalein. This is identified by the red color which it produces with alkalis.

In this laboratory the method worked expeditiously on aqueous solutions of soluble saccharin and ginger ale. The referee recommends<sup>4</sup> that the method be subjected to collaborative study during the coming year.

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 338.

<sup>2</sup> *Z. Nahr. Genussm.*, 20, 489 (1910).

<sup>3</sup> *Ann. Rpt. Dept. Agr. and Markets*, New York, 103 (1929).

<sup>4</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

## REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration,  
New York), *Referee*

Pursuant to the request of the Association, the referee sent out eight sets of alimentary paste samples to eight collaborators. Each set consisted of four subdivisions marked No. 1, No. 2, No. 3, and No. 4, respectively. Samples 1 and 2 were prepared from a good grade of semolina flour, to which a very small amount of coal tar dye had been added; viz, exactly 1 part of dye to 1 million parts of finished product. Samples 3 and 4 were ordinary egg noodles fortified with a small amount of vegetable color. The method suggested was a slight modification of the one used last year, *This Journal*, 18, 373 (1935).

The samples were colored as follows: No. 1 Sunset Yellow FCF, No. 2 Tartrazine, No. 3 Saffron, No. 4 Turmeric.

It is most regrettable that only three collaborators could find the necessary time to send in their results.

The reports of the collaborators as received follow:

|                          |   |
|--------------------------|---|
| M. L. Offutt<br>New York | No. 1. Sunset Yellow FCF<br>2 Tartrazine<br>3 Saffron<br>4 Turmeric |
| W. C. Woodfin<br>Atlanta | 1 Sunset Yellow FCF<br>2 Egg Yolk only<br>3 Saffron<br>4 Turmeric   |
| J. L. Hogan<br>New York  | 1 Sunset Yellow FCF<br>2 Tartrazine<br>3 Saffron<br>4 Turmeric      |

The following comments were made by the collaborators:

*M. L. Offutt.*—The method outlined presented no difficulty. A slight emulsion was noted when the solution was acidified prior to extraction with amyl alcohol.

*W. C. Woodfin.*—I believe it would be advisable to specify the proportion of acetic acid to be added to the aqueous solution after extracting the oil-soluble dyes. After separation of annatto from the ether solution, the test for turmeric can be made more pronounced if concentrated HCl is used instead of 1+1 acid.

*J. L. Hogan.*—Sample 1 did not offer any special difficulty. Sample 2 presented the most trouble because the dye seemed to be present in very small quantity. However, the 0.25 N HCl extract from the amyl alcohol solution, although faint in color, gave a positive wool dyeing, and as an additional test a coupling was applied to a portion of the solution with positive results. Samples 3 and 4 offered no trouble.

The reports of the collaborators indicate that the separation and identification of added coloring matter in alimentary products by the

suggested method was not difficult. If the observer bears in mind that Samples 1 and 2 contained 1 part of dye to 1 million of finished product and a charge of 500 grams could not possibly contain more than 0.5 mg. of dye, then the results obtained by the collaborators prove conclusively the adaptability of the method.

But as a matter of fact, the amount of alcoholic extract recovered is only about 500 cc.; this is approximately 5/7 of the total amount of solvent matter employed, which is equivalent to about 0.3 mg. of dye. This amount of sunset yellow FCF was not difficult to detect, but the same amount of tartrazine required very careful manipulation. It is therefore of interest to know that the tinctorial value of sunset yellow FCF is much greater than that of tartrazine.

The referee submitted two additional samples of colored macaroni to two collaborators. They were marked A and B. Sample A was colored with sunset yellow FCF, 1 part of color to 10 million of finished product. Sample B was colored with tartrazine, 1 part of color to 10 million of finished product. The report on these two samples follows:

|              |  |
|--------------|--|
| M. L. Offutt | <i>Sample A:</i> Sunset Yellow FCF     |
|              | <i>Sample B:</i> Tartrazine            |
| J. L. Hogan  | <i>Sample A:</i> Sunset Yellow FCF     |
|              | <i>Sample B:</i> Apparently Tartrazine |

The comments of the collaborators follow:

*M. L. Offutt.*—Sample A, although weak of color, was readily discerned. In Sample B the presence of color could only be proved by diazotization and coupling and later extracting the dye with a solvent, evaporating same and spotting the residue.

*J. L. Hogan.*—Sample A: Sunset yellow FCF, but in extremely small amount. Sample B: Resembles tartrazine, however its presence could not be directly established. Only by diazotization and coupling was its presence suspected.

This additional information indicates that the presence of sunset yellow FCF can be detected even in amounts as low as 0.03 mg., while the presence of tartrazine cannot definitely be established in less than 0.3 mg.

As a result of three years of collaborative work on this problem, it is the opinion of the referee that the suggested method for the separation and identification of added coloring matter in alimentary paste is an improvement on the present official method and therefore should displace same.

#### QUANTITATIVE ESTIMATION OF PONCEAU SX IN THE PRESENCE OF PONCEAU 3R

The method is based on the oxidation of the ponceau 3R in an alkaline medium, whereby it will be completely destroyed. Ponceau SX, on the

other hand, is much more resistant to this treatment and is oxidized only to a small extent. Since the amount of ponceau SX destroyed appears to be proportional under definite conditions, comparative results can be obtained:

*Experimental part.*—

Hydrogen peroxide—3% (10 vol.)

Sodium hydroxide—10%

Sulfuric acid—1+4

Ammonium acetate—1+1

Prepare a 1 per cent solution of the dye mixture. Titrate 20 cc. portions of the above solutions, using sodium citrate as a buffer. Mark a number of 300 cc. Erlenmeyer flasks with a suitable agent (crayon pencil or glass cutter) at volumes of 40 cc. and also 50 cc. Place a few glass beads into the flasks and into each pipet that amount of the dye solution, which is equivalent to 12–15 cc. of the standard 0.1N  $\text{TiCl}_3$ . Dilute with 50 cc. of water and add in the order named 8 cc. of hydrogen peroxide and 2 cc. of sodium hydroxide. Cover the flask with a short-stemmed funnel and boil the contents vigorously, over asbestos, until the volume reaches the 40 cc. mark. Add 25 cc. of water, 5 cc. of ammonium acetate, and 1 cc. of sulfuric acid, and boil again vigorously, until the 50 cc. mark is reached. Dilute the contents of the flask with 100 cc. of water, add 10 grams of sodium tartrate, and titrate with standard titanium trichloride until a colorless or yellowish solution is obtained. Multiply the number of cc. required to reduce the ponceau SX by 1.24, the result representing the true value of the latter dye. The difference between the computed figure and the total dye titration is due to ponceau 3R.

The results tabulated below were obtained by the method discussed.

| AMOUNT TAKEN  |                 |                | AMOUNT FOUND  |             |                        |                       |              |
|---------------|-----------------|----------------|---------------|-------------|------------------------|-----------------------|--------------|
| Ponceau<br>SX | Ponceau<br>+ 3R | Total<br>= dye | Ponceau<br>SX | Factor<br>× | PonceauSX<br>corrected | Ponceau3R<br>by diff. | Total<br>dye |
| gram          | gram            | gram           | gram          |             | gram                   | gram                  | gram         |
| 0.00438       | +0.1612         | =0.16558       | 0.00381       | ×1.24       | =0.00473               | +0.15676              | =0.16149     |
| 0.00876       | +0.1570         | =0.16576       | 0.00699       | ×1.24       | =0.00867               | +0.1524               | =0.16107     |
| 0.02191       | +0.1446         | =0.16651       | 0.01753       | ×1.24       | =0.02184               | +0.1445               | =0.16634     |
| 0.04380       | +0.1240         | =0.1678        | 0.03494       | ×1.24       | =0.04332               | +0.1243               | =0.16762     |
| 0.06571       | +0.1033         | =0.16901       | 0.05336       | ×1.24       | =0.06617               | +0.1022               | =0.16837     |
| 0.08764       | +0.08265        | =0.17029       | 0.07077       | ×1.24       | =0.08775               | +0.08264              | =0.17039     |
| 0.10517       | +0.06612        | =0.17129       | 0.08512       | ×1.24       | =0.10555               | +0.06542              | =0.17097     |
| 0.13146       | +0.04132        | =0.17278       | 0.10354       | ×1.24       | =0.12839               | +0.04421              | =0.17260     |
| 0.14899       | +0.02479        | =0.17378       | 0.11752       | ×1.24       | =0.14572               | +0.02740              | =0.17312     |
| 0.15775       | +0.01653        | =0.17428       | 0.12578       | ×1.24       | =0.15596               | +0.01668              | =0.17264     |

These results indicate that this method can be depended upon to give quantitative results if corrections are applied. It has the advantage of not being time-consuming, since a series of determinations can be completed in two hours.

The action of hydrogen peroxide and sodium hydroxide at first darkens ponceau 3R, later forms a deep brown precipitate, and ultimately de-



colorizes it. Ponceau SX by this treatment is not visibly affected. Less than 0.5 per cent of ponceau SX can be estimated in admixture with ponceau 3R. It is imperative that the volume of the solution after boiling should approach as closely as possible to the specified 40 cc. and 50 cc., otherwise the given factor will not apply.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the suggested method for the separation and identification of added coloring matter in alimentary paste be adopted as official and that the present official method be deleted.

(2) That the referee send out mixtures of ponceau SX and ponceau 3R to test the method submitted.

(3) That investigational work be undertaken for the quantitative separation of sunset yellow FCF from the other permitted dyes.

(4) That in the Chapter on Fats and Oils, a method for separation and identification of added coloring matter be included.

#### REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Department of Agriculture,  
Washington, D. C.), *Referee*

Last year the Referee on Metals in Foods recommended an ambitious program. That all the associate referees did not succeed in reaching the objectives laid out for them is not surprising. Some of the projects were new, and recommendations for adoption of methods were hardly to be expected.

#### ARSENIC

The associate referee made no formal report. The following comments on the progress of the work resulted from informal discussions. No work was done on the arsine distillation method of arsenic isolation and the molybdenum blue methods of arsenic determination. Some comparative collaborative work was done on especially refractory products by wet oxidative digestion, oxidation in closed systems, and dry ashing in open systems with fixatives as methods of sample preparation. The results of oxidation in closed systems are excellent, but the process involves special, more or less expensive apparatus, frequently evaporations of large volumes of solutions, possible limitations of size of sample, and necessity to work with dry material. These considerations limit the usefulness of methods involving oxidation in closed systems. As a substitute it has been suggested that arsenic may be determined in such refractory materials as

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

shrimp by wet digestion if a catalyst such as cupric sulfate is added and digestion is continued after clearing as in the usual Kjeldahl method. To avoid the difficulties inherent in the closed system oxidation, the associate referee also tried preparing the sample by an ashing process with fixatives in a manner similar to the fixation of sulfur compounds with nitric acid and magnesium nitrate, and found that this scheme worked quite well with inorganic arsenic but failed with the organic arsenic present in shrimp. The oxidative fixatives tried were mixtures of nitric acid, aluminum, and calcium nitrates. Possibly these reagents were not powerful enough for the organic arsenic. The referee suggests for trial next year the use of cerous nitrate in concentrated nitric acid. The referee will not make definite recommendations concerning sample preparation of difficult products at this time, but he stresses the fact that experience with tobacco and shrimp shows the necessity of adding to the methods already adopted a precautionary statement relative to the treatment of refractory samples.

Gross this year points out that efforts made to extend the upper range of the official Gutzeit method failed because, as in the case of large aliquots, proper consideration was not given to the effect of the sulfuric acid remaining after the digestion. No doubt this can be remedied by first neutralizing the sulfuric acid and then adding the full amount of hydrochloric acid. If this error is corrected, the study of extending the upper range of the official Gutzeit method can be continued. The lower range of the Gutzeit method could also be improved. The Lachele system of disks 10 mm. in diameter should be desirable for the 0-10 gamma range, where the official method is not fully effective. The information reported previously by Gross<sup>1</sup> relative to time and temperature of the arsenic reduction before starting the Gutzeit reaction should be incorporated in the revised *Methods of Analysis*, as well as the Mills<sup>2</sup> suggestion of acid-washed sand saturated with lead acetate and the permissive use of zinc pellets.

The associate referee points out that the tentative bromate method for arsenic must be carried out with strict observance of temperature and volume to avoid incomplete evolution of arsenic tribromide or titration of non-arsenical substances, the chief of which is SO<sub>2</sub>. Therefore the referee believes that a statement relative to checking the bromate results on important samples by a Gutzeit test on the distillate should be added to the present tentative method. One of the collaborators suggests the Gooch-Browning system of arsenic determination on the arsenic tribromide distillate instead of the bromate titration. This method has been tested with insecticides. The bromated distillate should also be suitable for a check by the Gooch-Browning method if it were preferred over the Gutzeit method. The next referee should look into the merits of this idea. Another sug-

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<sup>1</sup> *This Journal*, 16, 398 (1933).

<sup>2</sup> *Ibid.*, 18, 189, 506 (1935).

gestion is to test for sulfates in the final solution; if none are present, the bromate result could be considered correct.

### COPPER AND ZINC

Satisfactory methods for macro quantities of copper and zinc in foods and biological material have been adopted by this Association in previous years. The biochemists have developed three or four micro methods, but these have never been tested by collaborative work. The time seemed propitious, therefore, to invite the biochemists to cooperate and subject their methods to the test of collaboration, where inherent defects usually come to the surface. The fact that so many analysts took part augurs well for the success of the experiment.

By preparing the samples himself and removing the natural copper and replacing it with a known amount, the associate referee eliminated one variable and placed the emphasis on the isolation and determination of the copper. The results are good for a first effort on such quantities as 1.5 and 7.5 p.p.m. and constitute a firm basis for future efforts.

The question of the isolation of copper should receive special attention. Complex formation with extraction by a non-aqueous solvent might be better than precipitation for such micro quantities as are here involved, as illustrated by the success of dithizone as a lead extractant. Further work should show whether the carbamate method for the actual copper determination is better, or whether the dithizone "two-color" method might be applied to the best advantage. The principles of the lead determination involving measurements of light transmissions made with a photometer and suitable color filters should be applicable to the dithizone copper determination. Solving the interfering effects of the platinum metals, mercury, and possibly silver, bismuth, lead, and zinc should be possible, especially if more information on the stability of the various dithizone complexes with respect to hydrogen-ion concentration can be obtained.

Although the results obtained this year are good, considering that they are expressed as milligrams per liter and involve a multiplication factor of 40, the referee believes that a recommendation for adoption of a method should be postponed another year.

No reports have been made on zinc for a number of years. The referee believes that the subject is important enough to warrant the appointment of another associate referee with instructions to study micro zinc methods, including those involving the dithizone principle.

### FLUORINE

Last year the associate referee found that it is rather difficult to fix fluorine with lime, especially in the presence of carbohydrates, and that the more lime used the greater becomes the fluorine blank. The referee

then considered the inhibitive action of aluminum as in the Willard and Winter distillation,<sup>1</sup> and its strong interference in the peroxidized titanium reaction. These factors indicated a strong affinity between aluminum and fluorine. Dahle<sup>2</sup> has reported his studies on the factors that govern the fluorine distillation when aluminum is present, as well as when it is absent. Having first discovered a method for breaking the affinity of aluminum and fluorine, he then encouraged their union as an aid in ashing. He used aluminum nitrate, which supplies both oxygen for combustion and aluminum as a fluorine fixative. The results with the use of aluminum as a fixative are sufficiently encouraging to justify a recommendation of continuation of this line of investigation. Apparently with lime there are difficulties in the ashing, but less in the distillation, while aluminum generally works well during the ashing, but causes trouble in the distillation. Now that commercial lime with a fluorine content less than 10 p.p.m. has become available, one of the objections to the use of lime as a fixative has been largely removed. It is hoped that next year's work will demonstrate which one of these two fixatives should be selected.

Practically the only new development in suggested colorimetric fluorine methods last year was Dahle's blending of green copper nitrate and the yellow peroxidized titanium and matching the hues of the resulting mixtures in Nessler tubes against standards similarly prepared. The series of colors is through yellowish green, green, and blue-green. This matching of hues is similar to the hue-matching of the lead method and is preferred by some analysts. An advancement in the determination of the isolated fluorine was reported at the San Francisco meeting of the American Chemical Society.<sup>3</sup> It concerns the titration of the isolated fluorine in a buffer solution of about pH 3.75. The thorium titration generally fails when the fluorine in the aliquot taken is less than 0.1 mg. If titration in a buffer solution is really successful with quantities of fluorine less than 0.1 mg., then volumetric methods may in time further invade the realm of the colorimetric methods. The associate referee should follow this development closely.

In the referee's laboratory it is expected that the photoelectric apparatus being designed to measure minute differences of any color with the aid of proper color filters may demonstrate that the accuracy of the colorimetric peroxidized titanium method is about one gamma of fluorine. This sensitivity permits an evaluation of the different colorimetric fluorine procedures so that in due time the number may be reduced to one or two.

At this juncture the referee is in an unfortunate position with respect to the recommendation of a tentative method. No decision has been made on a procedure for preparing the sample. At present it depends largely

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1932).

<sup>2</sup> *This Journal*, 19, 313, 320 (1936).

<sup>3</sup> *Ind. Eng. Chem. Anal. Ed.*, 8, 6 (1936).

on the nature of the sample. The Willard and Winter method of fluorine isolation seems to be the only practical one, and Dahle's physico-chemical treatment of it has placed it on a scientific basis. After the fluorine has been isolated, titration of quantities above a certain minimum has been established, but the minimum may be reduced. As the referee is very familiar with the peroxidized titanium colorimetric method, he could urge its adoption as a tentative method, but he is not prepared to say that it is the best one available, because he is not equally familiar with other methods. It seems better to recommend further study on the preparation of sample, reduction of the minimum titratable quantity of isolated fluorine, and a re-evaluation of the available colorimetric fluorine methods even if it is not possible to get a general tentative method into the new edition of *Methods of Analysis*.

#### LEAD

The associate referee did not conduct collaborative experiments this year because the colorimetric dithizone and electrolytic methods were already well enough established in the spray residue field to serve as the basis for tentative adoption. Data were supplied last year, and more could be supplied this year, if necessary. It was considered more important to learn enough of the mechanics of the dithizone reaction to apply the method to the determination of lead in any food or biological material and in any reasonable micro amount. It was also felt that the labor of the preparation of standards could be reduced by the development of suitable apparatus, even if the problem of the interference of bismuth had to be somewhat neglected.

In order to plan future work a flow sheet (Fig. 1) was prepared to show the methods of sample preparation, separation and isolation of lead, and the final determination as suggested by workers in this field. Some of the broken lines represent the so-called rapid methods, used particularly in spray residue work on apples. Here some accuracy is sacrificed for speed. Other broken lines represent that part of past standard practice that has been useful in the development of the newer methods. Some of the indicated methods are still used to a limited extent. The solid lines represent the procedures which the referees believe will result ultimately in official methods. The ranges of determined lead inserted in the proper places represent the present ideas of the referees.

The referee considers the best procedure for lead isolation to be the dithizone extraction wherever it is possible to use it. It fails only in the comparatively few instances where colloids develop emulsions or precipitates adsorb lead when the lead solution is made ammoniacal. Citrates help to prevent such precipitates, but when the samples contain too much calcium phosphate or much smaller quantities of magnesium phosphate, precipitates cannot always be prevented. In such cases the precipitates

are dissolved with acid and the lead is separated by the sulfide precipitation process. The optimum hydrogen-ion concentrations for separations and determinations are given in Fig. 1.

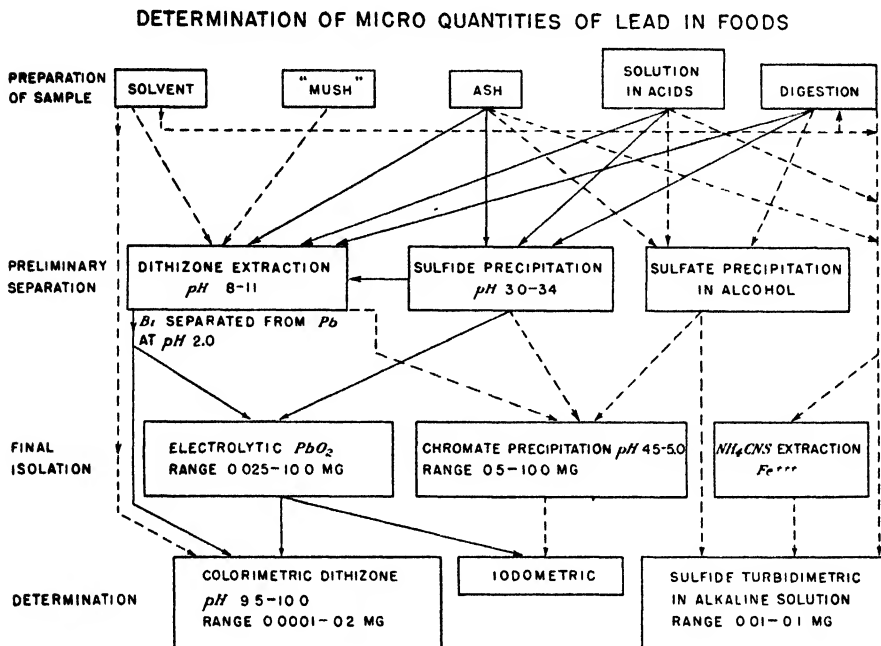


FIG. 1

Interferences and ways of avoiding them are not shown, with the exception of the Willoughby<sup>1</sup> method for separating lead and bismuth, as indicated by the two optimum hydrogen-ion concentrations for dithizone extraction. Winter et al.<sup>2</sup> claim that a separation can be made in a quite alkaline medium of pH 11-12. Wichmann and Clifford<sup>3</sup> show how the interference of tin can be avoided by volatilization as stannic bromide, and how bismuth iodide can be extracted by ethyl acetate and thus separated from lead. These methods for the removal of interfering elements are complicated, and a simplification would be welcome.

Electrolytic separation followed by iodometric determination of lead has been very successful when the quantity of lead exceeds 0.05 mg. The colorimetric dithizone (0.0001-0.2 mg. Pb) and electrolytic methods, therefore, supplement each other with some overlapping, which is quite convenient at times as an independent check. The sulfate method of preliminary lead separation is used to a small extent. The color chemists in America and England seem to prefer it. It is believed that the chromate

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 7, 285 (1935).

<sup>2</sup> *Ibid.*, 265.

<sup>3</sup> *This Journal*, 18, 815 (1935).

isolation of micro quantities of lead is obsolete, due mainly to its definite though small solubility. Fundamentally, turbidimetric are less reliable than gravimetric, volumetric, or colorimetric methods, due to troubles concerning particle size or the formation of precipitates other than the one desired. In spite of these well-known objections, however, the turbidimetric sulfide lead method continues to be used. This may be largely due, in this country at least, to the development of the photo-electric method of comparison.<sup>1</sup> No doubt some satisfactory results can be obtained with it. The method will probably continue to be used for some purposes, but the referee believes it will gradually be displaced.

On the basis of Clifford's fundamental work on the general colorimetric dithizone method for very minute quantities of lead, the satisfactory results of the special colorimetric dithizone method as applied to apples, and the wide applicability of the electrolytic method to all sorts of material where 0.05 mg. or more of lead can be isolated, the referee believes he is justified in recommending that these two methods be adopted tentatively by this Association. Some well-planned collaborative work next year by the skilled analysts that are now available should furnish sufficient data to justify speedy official adoption. Completion of the associate referee's work on the general colorimetric dithizone method<sup>2</sup> is so recent that there has been no time to prepare a tentative description of these methods for presentation to the Association at this meeting. The referee believes that part of the flow sheet, with methods of sample preparation, isolation, and determination should appear in the next edition of *Methods of Analysis* in the section on Metals in Foods. Special methods of sample preparation, isolation, and determination applicable to particular products should be included in this section for the next edition, but they may logically be transferred to their respective product sections in future revisions. The products especially recommended for further study are oils and fats, baking powders, and preparations of fish and meat samples for analysis.

#### MERCURY

Winkler's<sup>3</sup> titrimetric method for mercury has just been published. This method is similar in some respects to the Willoughby<sup>4</sup> titrametric method for lead. The most serious interference likely to be encountered in the determination is material amounts of copper, but Winkler has shown how the copper may be separated from mercury when it is necessary. The associate referee found it necessary to destroy organic matter completely, which obviously must be done by wet methods under a reflux. As the destruction of the organic matter is laborious, some procedure should be devised to extract mercury without complete oxidation of

<sup>1</sup> *This Journal*, 17, 141 (1934).

<sup>2</sup> *Ibid.*, 19, 130 (1936).

<sup>3</sup> *Ibid.*, 18, 638 (1935).

<sup>4</sup> *Loc. cit.*

organic matter. Dithizone is extremely sensitive to oxidizing agents, therefore the simple device of having some hydroxylamine salt present to reduce excess oxidizing agents can perhaps be used to advantage in the determination of other metals. Winkler reports a few good collaborative results. On the basis of his own work the method seems to have an extreme sensitivity of about one gamma. Fischer and Leopoldi<sup>1</sup> recently published a paper describing a titrametric dithizone method based on similar principles, as well as a colorimetric method for the determination of mercury of about the same sensitivity. Ordinarily the referee would desire more collaborative work as the basis for a recommendation of even a tentative method, but he will make an exception and recommend a tentative status for Winkler's method in order to get a mercury method into the new edition of *Methods of Analysis*. It appears to be based on sound principles, and it is the most practical method for the micro determination of mercury in foods now available.

#### SELENIUM

The associate referee's account of the present uncertainty of selenium methods shows that analysts are not in agreement as to the best method for sample preparation and determination. They do agree on the method of isolation. Owing to these differences it is not possible at the present time to recommend any methods for adoption.

#### DITHIZONE METHODS IN GENERAL

Hellmut Fischer's<sup>2</sup> recent article on the absorption curves of dithizone and its complexes in carbon tetrachloride is very opportune and should be called to the attention of American chemists. His curves for dithizone and its lead complex closely parallel the data reported by Clifford and Wichmann.<sup>3</sup> His curves for the copper, silver, mercury, cadmium, zinc, tin, thallium, cobalt, and nickel complexes present useful information to the chemist interested in the development of new dithizone methods for the determination of these metals. The absorption maxima show the nature of the color filters required, and therefore save much time. The maximum absorptions for most of these metal complexes are at wave lengths 500–560  $m\mu$ . Silver has a maximum at 462, mercuric mercury from acid solution one at 490, and cupric copper from alkaline solution one at 450  $m\mu$ . The referee suggests that analysts should not overlook the possibilities of distinguishing by optical methods those metals having absorption maxima from 450 to 500 from those in the 500–560  $m\mu$  range.

In the paper published by Clifford and Wichmann<sup>4</sup> are shown some significant sigmoid curves showing the equilibrium between the lead-dithizone complex and hydrogen-ion concentration. These equilibria can

<sup>1</sup> *Z. anal. Chem.*, 103, 241 (1935).

<sup>2</sup> *Wiss. veröffentl. Siemens-Werken*, 14, 241 (1935).

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *Loc. cit.*



be shifted to the left or right according to the amount of excess dithizone. The referee desires to call attention to the relationship of the lead-dithizone equilibrium curves and similar hypothetical curves for bismuth and tin. The only clue to the shape of the bismuth and tin equilibrium curves is found in the papers of Willoughby,<sup>1</sup> Winter,<sup>2</sup> and Fischer.<sup>3</sup> Willoughby separates bismuth from lead by extracting their aqueous solutions at a pH of 2.0 with a chloroform solution of dithizone, while the other two writers extract bismuth (Winter also separates tin) from non-aqueous solutions of their dithizone complexes with aqueous solutions of pH 11-12. This is taking advantage of the variation in stability of the dithizone complexes towards hydrogen-ion concentration. The bismuth equilibrium curve is therefore believed to be a double sigmoid somewhat similar to the lead curve, but with the maximum shifted one or two pH units to the left. According to very meager information at present, the equilibrium between stannous tin and hydrogen-ion concentration may be similar to the bismuth relationship, but shifted slightly to the left. No doubt the other dithizone metals also possess definite equilibrium curves, complicated perhaps in the case of those metals that exist in both keto and enol dithizone combination.<sup>4</sup> The acquisition of information with reference to these equilibria is highly desirable, because if it were available and added to the present knowledge of complex ion formation, the problem of interference would be greatly simplified, which in turn would facilitate the development of new methods. The Association needs assistance in advancing this project, and to this end the referee suggests that the preparation of the curves would be good subjects for theses in the colleges.

#### RECOMMENDATIONS\*

It is recommended—

(1) That work on the arsine distillation method of arsenic isolation, the molybdenum blue methods of arsenic determination, and the ashing with fixatives method of sample preparation be continued.

(2) That a statement embodying the extra precautions to be taken in the sample preparation of specially refractory materials be added to the official Gutzeit method.

(3) That efforts designed to increase the range of the Gutzeit method in both directions be continued.

(4) That certain changes in the official Gutzeit method designed for its improvement, and discussed briefly by the referee, be added to the official Gutzeit method. That a warning concerning the necessity of strictly observing the conditions of temperature and volume, and a sug-

<sup>1</sup> *Loc cit*

<sup>2</sup> *Loc cit*

<sup>3</sup> *Angew. Chem.*, 47, 90, 685 (1934)

<sup>4</sup> Fischer, *loc cit*

\* For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

gestion for checking the bromate results, be likewise added to the tentative bromate method.

(5) That work on the determination of micro amounts of copper be continued. Part of this work should be devoted to the dithizone method.

(6) That the work on zinc be separated from copper and placed under the direction of a new associate referee.

(7) That work on the determination of fluorine be continued.

(8) That the colorimetric dithizone and electrolytic methods for the determination of lead be made tentative, and that studies on lead methods be continued.

(9) That the dithizone titrimetric method for mercury be made tentative and that studies on mercury be continued.

(10) That work on selenium methods be continued.

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No formal report on arsenic was given by the associate referee.

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## REPORT ON COPPER

By E. J. COULSON (U. S. Bureau of Fisheries, Charleston, S.C.),  
*Associate Referee*

Inasmuch as the present tentative method for the determination of copper in foods has been shown by a previous referee to give unsatisfactory results when small amounts of copper are present,<sup>1</sup> it was recommended by the Referee on Metals in Foods that in this year's study especial attention be paid to the colorimetric methods used by biochemists, and that men familiar with such methods be invited to collaborate in the study.

Letters of inquiry were mailed to biochemists who are known to be particularly interested in the anemia problem and to others also known to be interested in micro copper methods. The replies indicated that two methods, each with various individual modifications, are most commonly used. These, in order of their indicated preference, are the carbamate method<sup>2,3</sup> and the Biazzo<sup>4,5</sup> method. The nature of the replies also revealed that an increasing amount of interest is being shown in the chromotropic method.<sup>6,7</sup>

The use of sodium diethyldithiocarbamate, which gives a brown precipitate of the normal copper salt of diethyldithiocarbamic acid with solutions containing copper, was first proposed for the colorimetric determination of copper by Callan and Henderson.<sup>2</sup> McFarlane<sup>3</sup> modified

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<sup>1</sup> Mehurin, *This Journal*, 16, 330 (1933).

<sup>2</sup> Callan and Henderson, *Analyst*, 54, 650 (1929).

<sup>3</sup> McFarlane, *Biochem. J.*, 26, 1022 (1932).

<sup>4</sup> Biazzo, *Ann. chim. applicata.*, 16, 2 (1928).

<sup>5</sup> Elvehjem and Lindow, *J. Biol. Chem.*, 81, 435 (1929).

<sup>6</sup> Ansbacher, Remington, and Culp, *J. Ind. Eng. Chem. Anal. Ed.*, 3, 314 (1931).

<sup>7</sup> Sheets, Pearson, and Gieger, *Ibid.*, 7, 109 (1935).

the method by extracting the organic salt from its aqueous solutions with amyl alcohol, thereby improving the sensitiveness of the test and increasing the range of concentration within which the method can be applied.

In the Biazzo method as modified by Elvehjem and Lindow<sup>1</sup> the green complex copper-thiocyanate-pyridine compound formed by the addition of small amounts of potassium sulfocyanate and pyridine to the weakly acid copper solution is extracted with chloroform and compared in a colorimeter with a standard copper solution prepared in the same manner.

The chromotropic method is a titration procedure and depends upon the formation of an intense permanganate color resulting from the union of one cupric ion with two molecules of the nitrosochromotropic salt (1, 8-dihydroxy-2-nitroso-3, 6-naphthalenedisulfonic acid) in an ammoniacal solution. As soon as an excess of the yellowish brown nitroso dye is present, the permanganate color changes to brown. Sheets, Pearson and Gieger<sup>2</sup> have recently modified this titration procedure in order to make the determination of the end point more easily distinguished.

The carbamate and Biazzo procedures can both be applied directly to the ash of many foods without the separation of the copper from the other constituents of the ash; however, in materials such as liver, blood, etc., the relatively large quantities of iron interfere and must be removed or inactivated in some manner before the color development. Likewise in materials such as milk and bones, the ash contains large amounts of calcium phosphate which precipitates in an alkaline solution and from which, according to McFarlane; Ansbacher, Remington and Culp; and Conn, Johnson, Trebler and Karpenko<sup>3</sup> the copper cannot be quantitatively recovered. One means of separating copper from interfering substances is by hydrogen sulfide precipitation. This procedure has been criticized by Walker<sup>4</sup> as being of doubtful value for such small quantities, but the associate referee believes with Ansbacher, Remington, and Culp that minute quantities of copper can be quantitatively separated from solutions in this manner if proper precautions are taken. In the present investigation the copper was precipitated in a small volume (20–25 cc.) of .3 *M* hydrochloric acid ( $pH=0.5$ ), and the tightly stoppered tube was allowed to stand overnight. Filtration was carried out in a special porous bottom crucible, and the cupric sulfide was redissolved with nitric acid, the dissolved precipitate being collected in the original precipitating tube. This procedure effects a separation of the copper from the metals of the third group and from mercury. Other metals of the second group do not interfere unless present in more appreciable quantities than are ordinarily encountered in foods.

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<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *Ind. Eng. Chem. Anal. Ed.*, 7, 15 (1935).

<sup>4</sup> *This Journal*, 13, 426 (1930).

Two solutions were prepared for collaborative study this year. Solution 1 contained the ash of dried whole milk and Solution 2 contained the ash of dried spinach. The ash of both substances was dissolved in sufficient hydrochloric acid to make the final concentration equivalent to .3 M HCl and each cubic centimeter of solution contained the ash from 1 gram of material. The copper was removed by two precipitations with hydrogen sulfide carried out in the following manner: The solution was heated to boiling, and a rapid stream of hydrogen sulfide was bubbled through until it cooled to room temperature, after which the outlet tube of the precipitating flask was closed and allowed to stand overnight under the pressure of hydrogen sulfide from the generator. The following morning the solution was filtered by suction through porous bottom filter crucibles and hydrogen sulfide was removed from the filtrate by boiling. To Solution 1 was added sufficient copper standard to make the final concentration of copper equivalent to 1.53 mg. of copper per liter, and Solution 2 contained 7.65 mg. per liter. All dilutions were made with water that had been redistilled from glass.

These prepared solutions were sent out to 14 collaborators with the request that copper be determined by both the carbamate and Biazzo methods following precipitation and filtration of the copper by hydrogen sulfide as described in Method I, with any additional time they cared to give to be employed in the trial of Methods II and III or in estimation by their own favorite method. A small quantity of Bureau of Standards analyzed copper, with directions for the preparation of copper standard solution, was furnished to each collaborator.

These methods of separation of the copper sulfide from the solutions were outlined and were as follows:

#### SEPARATION

*Method I (Filtration).*—Transfer an aliquot (20–25 cc.) of the test solution into a large Pyrex test tube. Heat the solution by placing the test tube in a beaker of boiling water; remove from the hot water and saturate while cooling with washed hydrogen sulfide gas for 15 minutes. During the last 5 minutes place the test tube in a beaker of cold water. Pass the hydrogen sulfide gas into the solution through a capillary glass tube (a 4 or 5 mm. glass tube drawn out to a capillary of about 1 mm. outside diameter). Turn on the gas before the delivery tube is put into the solution and turn it off after the tube is removed to prevent possible loss of the solution on the inside of the tube. When saturation is complete, rinse the delivery tube with 0.5–1.0 cc. of freshly prepared hydrogen sulfide water (containing 25 cc. of concentrated hydrochloric acid per liter) when removing it from the solution. Stopper the tube tightly and allow to stand overnight.

Filter by gentle suction through a small crucible with porous bottom (Central Scientific Co. No. 3410 AX filtering crucibles, Berlin, porosity 1) or through a micro-Jena glass sintered filter. Rinse (but do not police) the test tube once with 1–2 cc. of the freshly prepared acidified hydrogen sulfide water and pour through the filter, taking care that the copper sulfide precipitate does not remain in contact with the air any appreciable length of time during this filtration. When filtration is com-

plete, select a filter flask into which the large test tube will fit so that the outlet tube of the crucible holder drains into the test tube in which the precipitation was carried out. Place the crucible containing the copper sulfide precipitate into the crucible holder (of the type in which the filtrate does not come into direct contact with rubber; see Central Scientific No. 3446-A), and fill the crucible with hot concentrated nitric acid. After allowing to stand a few minutes, apply a gentle suction and wash several times with copper-free distilled water. Transfer the nitric acid solution of the copper into a 100 cc. beaker and evaporate cautiously until only one or two drops of the acid remain. Take up the copper salt with a small amount (10–15 cc.) of copper-free water and determine its copper content.

*Method II.*—Shake out the precipitated copper sulfide with chloroform, and proceed with precipitation with hydrogen sulfide and collection of the copper sulfide in chloroform essentially as directed by Gebhardt and Sommer.<sup>1</sup>

*Method III.*—Follow the centrifugation procedure described recently by Conn, Johnson, Trebler and Karpenko.<sup>2</sup>

The collaborators were directed to follow the regular procedures in estimating the copper by the carbamate and Biazzo methods with one exception—the substitution of iso-amyl alcohol for the normal amyl alcohol in the carbamate method—as suggested by Thatcher.<sup>3</sup>

#### ESTIMATION

*Biazzo Method.*—Transfer the copper solution obtained in Method I (or the neutralized copper solution from Method II) to a 25 cc. glass-stoppered volumetric flask or separatory funnel. Add enough 1 *N* NaOH to make the solution just alkaline to phenolphthalein. Then add 1 cc. of glacial acetic acid, 1 cc. of a 10 per cent solution of potassium thiocyanate, and 10 drops of pyridine (recently distilled) in the order given, with slight shaking after each addition. Finally add 5 cc. of chloroform, accurately measured, and make up the volume to 25 cc. with distilled water. Shake thoroughly to allow the chloroform to take up the green copper-thiocyanate-pyridine compound. Remove the water portion and use the chloroform solution for the colorimetric comparison with a standard in a suitable colorimeter.

Prepare the standard by adding 1 cc. of glacial acetic acid to an appropriate amount of copper standard solution (as indicated by the depth of color developed in the unknown) and proceeding as with the unknown.

**NOTE.**—In the Gebhardt and Sommer modification the standard should contain 1 cc. of concentrated nitric acid and be neutralized with dilute sodium hydroxide as directed for the unknown in the procedure.

*Carbamate Method.*—Add concentrated ammonium hydroxide to the copper solution obtained in Method I, until alkaline to litmus. Transfer to a 25 cc. glass-stoppered cylinder or separatory funnel and add 1.0 cc. of 1 per cent aqueous solution of sodium diethyldithiocarbamate, and then 10 cc. of iso-amyl alcohol (redistilled at 129–131° C.), carefully measured. Stopper the flask and shake for about 2 minutes. Decant the amyl alcohol layer containing the colored copper salt and filter through a 2.5–3 mm. paper to remove traces of moisture, or transfer the alcohol layer to clean test tubes and centrifuge to clear (the associate referee prefers the latter). Compare with the standard in a suitable colorimeter.

Prepare the standards by adding 2 drops of nitric acid and proceeding exactly as directed for the unknown, beginning with “make alkaline to litmus with  $\text{NH}_4\text{OH}$ .” Read the unknown against a standard which nearly matches it in color.

<sup>1</sup> *J. Ind. Eng. Chem. Anal. Ed.*, 3, 24 (1931).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *J. Am. Chem. Soc.*, 56, 4524 (1933).

## RESULTS

Analysis of the solutions by the associate referee, following Method I and the Biazzo procedure, gave the following results, expressed as mg. Cu per liter: 1.77 and 1.62 for Solution 1, and 7.72 and 7.50 for Solution 2. Method I and the carbamate procedure gave the following results: 1.52 and 1.54 for Solution 1, and 7.37 and 7.30 for Solution 2.

The results from the thirteen collaborators who replied are shown in Tables 1, 2, and 3.

TABLE 1.—*Collaborative results by Biazzo method*  
(Expressed as mg. Cu per liter)

| COLLABORATOR  | METHOD I                |                                  | METHOD II                    |                              | METHOD III   |                      |
|---|-------------------------|----------------------------------|------------------------------|------------------------------|--------------|----------------------|
|   | SOLN. 1*                | SOLN. 2†                         | SOLN. 1                      | SOLN. 2                      | SOLN. 1      | SOLN. 2              |
| A. H. Johnson and<br>Lillian Conn<br>Baltimore, Md.   | 1.49<br>1.58            | 6.40<br>6.55                     |                              |                              | 1.42<br>1.44 | 7.43<br>7.56<br>7.02 |
| C. A. Elvehjem and<br>M. O. Schultze<br>Madison, Wis. | 1.977<br>2.180<br>2.624 | 7.08<br>8.09<br>8.00             |                              |                              |              |                      |
| W. E. Krauss and<br>R. G. Washburn<br>Wooster, Ohio   | 2.348<br>2.036<br>2.123 | 8.735<br>8.838<br>8.601<br>7.059 |                              |                              |              |                      |
| R. W. Titus and<br>E. C. Teut<br>Marysville, Ohio     | 1.99<br>1.96<br>1.94    | 7.94<br>7.40<br>7.53             | 1.73<br>1.68<br>1.60<br>1.41 | 8.27<br>8.15<br>8.11<br>7.38 |              |                      |
| G. T. Lewis<br>Emory University<br>Georgia            | 2.00<br>2.14<br>1.98    | 7.50<br>7.39<br>7.19             |                              |                              |              |                      |
| D. L. Drabkin<br>Philadelphia, Pa.                    | 1.78<br>1.67            | 7.10<br>7.33<br>8.43             | 1.61<br>1.59                 | 6.92<br>6.32<br>5.62         |              |                      |
| C. L. Smith<br>Charleston, S. C.                      | 1.70<br>1.62<br>1.58    | 6.61<br>7.50<br>7.38             |                              |                              |              |                      |

\* Solution 1 contained 1.53 mg. Cu per liter.

† Solution 2 contained 7.65 mg. Cu per liter.

TABLE 2.—*Collaborative results by carbamate method*  
(Expressed as mg. Cu per liter)

| COLLABORATOR   | METHOD I                         |  | METHOD II |              | METHOD III   |                                  |
|--|----------------------------------|--|-----------|--------------|--------------|----------------------------------|
|  | SOLN. 1*                         | SOLN. 2†   | SOLN. 1   | SOLN. 2      | SOLN. 1      | SOLN. 2                          |
| A. H. Johnson and<br>Lillian Conn                      | 1.94<br>1.45                     | 7.00<br>7.21<br>6.60                               |           |              | 1.82<br>1.63 | 6.58<br>6.58<br>6.26             |
| C. A. Elvehjem and<br>M. O. Schultze                   | 1.04<br>.80<br>.53               | 6.38<br>7.00<br>6.54                               |           |              |              |                                  |
| W. E. Krauss and<br>R. G. Washburn                     | 1.865<br>1.814<br>1.956<br>1.956 | 8.536<br>8.868<br>8.601<br>7.602<br>7.459<br>7.286 |           |              |              | 6.864<br>7.130<br>6.994<br>6.806 |
| R. W. Titus and<br>E. C. Teut                          | 1.54                             | 7.92<br>7.68<br>8.23                               |           |              |              |                                  |
| G. T. Lewis  | 1.27<br>1.57                     | 10.29<br>6.43<br>7.16                              |           |              |              |                                  |
| D. L. Drabkin  | 1.48<br>1.36<br>1.22             | 7.40<br>8.12<br>6.24                               |           | 7.48<br>7.36 |              |                                  |
| L. S. Palmer and<br>J. W. Nelson<br>St. Paul, Minn.    | 1.73<br>1.77                     | 8.00<br>8.49<br>8.49                               |           |              |              | 7.40<br>7.43                     |
| C. L. Smith  | 1.79<br>1.92<br>1.79             | 8.48<br>7.76                                       |           |              |              |                                  |
| W. S. Ritchie and<br>E. M. Doerpholz<br>Amherst, Mass. | 1.688<br>1.709                   | 9.662<br>9.662                                     |           |              |              |                                  |
| B. R. Fudge<br>Lake Alfred, Fla.                       | 1.666<br>1.684<br>1.724          | 8.533<br>8.572<br>8.642                            |           |              |              |                                  |

\* Solution 1 contained 1.53 mg. Cu per liter.

† Solution 2 contained 7.65 mg. Cu per liter.

TABLE 3.—*Collaborative results by other methods*  
(Results expressed as mg. Cu per liter)

| COLLABORATOR   | SOLUTION 1*              | SOLUTION 2†                    | METHOD   |
|--|--------------------------|--------------------------------|--|
| A. H. Johnson and<br>Lillian Conn                        | 1.44<br>1.36             | 6.12<br>6.12<br>5.94           | Modified carbamate   |
| C. A. Elvehjem and<br>M. O. Schultze                     | 1.69<br>1.61             | 8.33<br>8.05                   | Dithizone  |
| W. E. Krauss and<br>R. G. Washburn                       | 1.62<br>1.68             | 7.675                          | Carbamate—copper precipitated<br>from calcium phosphate and iron<br>by ammonia precipitation   |
| D. L. Drabkin  | 1.52<br><br>1.82         | 8.11<br>8.66<br>8.94<br>8.44   | Biazzo method { iron inactivated by<br>pyrophosphate and<br>copper separated from<br>calcium phosphate by<br>ammonia precipitation<br>Carbamate method |
| Olive Sheets and<br>an assistant<br>State College, Miss. | 1.77<br><br>2.62<br>2.62 | 7.39<br>9.12<br>8.582<br>8.740 | Chromotropic   |
| Margaret Ohlson<br>Ames, Iowa                            | 2.054<br>1.898           | 8.320<br>7.384                 | Chromotropic   |
| R. B. Dustman and<br>Mr. Brown<br>Morgantown, W. Va.     | 2.360<br>2.360<br>2.390  | 8.125<br>8.041<br>8.042        | Chromotropic   |
| L. S. Palmer and<br>J. W. Nelson                         | 1.95<br>1.20<br>1.60     | 7.27<br>8.12<br>8.40           | Chromotropic, after filtration by<br>Method I  |
| B. R. Fudge  | 1.714<br>1.780           | 8.572<br>8.572                 | Chromotropic, after filtration by<br>Method I  |

\* Solution 1 contained 1.53 mg. Cu per liter.

† Solution 2 contained 7.65 mg. Cu per liter.

#### COMMENTS OF COLLABORATORS

*A. H. Johnson and Lillian Conn.*—If carefully carried out, there is nothing to choose between the Biazzo and carbamate methods of colorimetric comparison or between the centrifugation and filtration methods of copper precipitation in point of view of accuracy for determination of amounts of copper above 1 p.p.m. For precipitation of copper, this laboratory favors the centrifugation method, probably because we have had more experience with it and because it involves no transfer of precipitate. This makes it simpler and there is less chance of contamination or loss.



For colorimetric comparison we prefer the carbamate method. It is more sensitive, which makes it possible to use smaller samples. We have not had as much experience with the Biazzo method and found the color more difficult to compare, especially the sample containing the smaller amount of copper. However, results seemed to compare favorably with those obtained by the carbamate method.

*C. A. Elvehjem and M. O. Schultze.*—Upon neutralization of the nitric acid solution with ammonium hydroxide to alkaline reaction, a fine white precipitate was formed. During the last steps of the procedure this precipitate undoubtedly adsorbed some of the carbamate-copper complex, as it appeared brown upon centrifuging. This probably accounts for the low results obtained and the poor agreement between aliquots. The results obtained with the Biazzo and the carbamate methods of copper determination following separation by filtration do not warrant their recommendation in the present form for general use. The Biazzo method under the conditions prescribed gives better results, especially if the amount of Cu to be determined is not less than 0.10 mg. We would urge substitution of ammonium hydroxide for the sodium hydroxide used, since the former is so readily purified.

*W. E. Krauss and R. G. Washburn.*—The reported results vary more than we usually find to be the case by starting at scratch with a given sample and running triplicates, with one recovery.

*R. W. Titus and E. C. Teut.*—No difficulty was encountered in filtering the prepared samples, but believe we might have had trouble with a regular milk ash particularly were it contaminated with tin. With reference to the use of nitric acid, we wonder whether it is necessary to use such a large quantity in order to dissolve the sulfide, since a considerable amount of time is required to evaporate this nitric acid and then there is always a question of copper contaminates in the reagents. In the chloroform extraction method there is, of course, the advantage of speed, but there is also a possibility of drawing off some of the chloroform solution due to poor separation or attempting to draw too closely. With reference to the carbamate method, we believe the depth of color for a small amount of copper and the clearness of the alcohol solutions are decided advantages.

*G. T. Lewis.*—The Biazzo method at least gave consistent results on both samples while the carbamate method did not. The variation from 7.50 to 7.19 in the Biazzo method on Solution 2 represents a difference of only 0.3 mm. in the colorimeter reading, which is about as accurate as any colorimetric method is in my hands.

*L. S. Palmer and J. W. Nelson.*—I believe the report indicates that the better duplicates are obtained by the carbamate method when employing the same procedure than by the chromotropic method.

*D. L. Drabkin.*—Directions for redistilling nitric acid should be explicitly given. If the nitric acid were contaminated with copper, the analysis would suffer. Cork stoppers should be specified in Method I; rubber is notoriously contaminated. Pyridine should not only be freshly redistilled, but should be collected at the temperature noted. The direction which calls for "10 drops" of pyridine in the Biazzo method is definitely poor. My pipet requires 25 drops for full color development. The directions should be changed to 0.5–1.0 cc.; 0.5 cc. is probably sufficient in most cases. The length of time the nitric acid is to be allowed to stand in contact with sulfide in the crucible, before filtration (Method I) should be specified. The amount of water necessary to thoroughly wash should be specified. In copper separation Method II the directions definitely suggest that samples not greater than 10 cc. be employed, so that proper shaking out with chloroform in a 25 cc. flask may be carried out. . . . Does not this cramp this technic? It certainly forces one to read very dilute solutions with Solution 1, especially objectionable with the Biazzo method. Since this seems to be the simplest of the copper sulfide separation pro-

cedures, it may be worth while to try extracting with, let us say, 10 cc. quantities of chloroform in 50 cc. flasks, thus permitting the use of larger samples. There cannot be the slightest doubt as to the superiority of the carbamate technic over the Biazzo colorimetrically, when small amounts of copper (.02-.06 mg.) are involved. The color is far more intense, and the separation of amyl alcohol and transfer to the colorimeter cup is far easier than that of the chloroform in the Biazzo method.

### DISCUSSION

Insufficient data are presented to draw conclusions as to the accuracy of the procedures other than Method I, and these results can hardly be said to be satisfactory. Unfortunately an insufficient amount (about 225 cc.) of the solutions may have been sent to allow the collaborators to become familiar with the technic before making the determinations. Whether or not this would have influenced the results is not known. The 25 cc. aliquots of Solution 1 that were taken for analysis contained only 0.0382 mg. of copper, and, as is well known, the accurate estimation of this small amount of copper in the presence of interfering substances is not easy. It is the opinion of the associate referee that these procedures show promise, and it is believed that with the experience gained in this year's study further revision and refinement in the procedures preceding the development of the colors in the two methods can be made.

A majority of the collaborators expressed preference for the carbamate procedure because of its more intense color when small quantities of copper are involved and because the separation of the iso-amyl alcohol and transfer to the colorimeter cup is far easier than that of the chloroform in the Biazzo method.

In Table 3 are shown the results of copper determinations by the usual procedures in use in the laboratories of the different collaborators. It is of interest to note that two of the collaborators (Krauss and Drabkin) obtained good results with an ammonia precipitation procedure for the separation of copper from calcium phosphate and iron, a procedure that has been criticized as giving low results by Ansbacher et al.,<sup>1</sup> McFarlane,<sup>2</sup> and recently by Conn et al.<sup>3</sup> Of interest also in this table are the results obtained by collaborators Elvehjem and Schultze with the "dithizone" method of Fischer.<sup>4</sup> These collaborators state that they have been using this procedure since April 1934 and are highly pleased with the results. They have kindly furnished an outline of their procedure and since it differs from that proposed by the referee last year<sup>5</sup> and does not appear in English or American journals, a brief summary of their procedure as used on the test solutions is given here.

To an aliquot containing 0.008-0.020 mg. of copper add 20% (by volume) HCl at the rate of 2 cc. for each 10 cc. of the ash solution used. Extract the copper

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *Loc. cit.*

<sup>4</sup> *Z. angew. Chem.*, 47, 90, 685 (1934).

<sup>5</sup> Mehurin, *This Journal*, 18, 192 (1935).

with 2 cc. portions of the dithizone reagent (in  $\text{CCl}_4$ ) in a separatory funnel until the reagent is no longer discolored. Wash the extract again in separatory funnels, in succession with 2%  $\text{H}_2\text{SO}_4$  and twice with  $\text{NH}_4\text{OH}$  (1+200). Finally wash with 1%  $\text{H}_2\text{SO}_4$  containing a little  $\text{SO}_2$ . After each separation in the funnel wash the remaining aqueous solution with 0.5-1 cc. of pure  $\text{CCl}_4$  to insure complete extraction. Filter the extract through a small filter paper (2.5-3 cm.) into a graduated centrifuge tube. Wash the filter with  $\text{CCl}_4$  and make the solution up to volume. It is advisable to cover the  $\text{CCl}_4$  solution at this juncture with a layer of 1%  $\text{H}_2\text{SO}_4$  containing a little  $\text{SO}_2$  and to keep it in a dark desk until all samples and the standard have been developed, then to make the colorimetric comparison at once.

During the past year the dithizone reagent has also been recommended by Sylvester and Lampitt<sup>1</sup> for the extraction of copper from the ash of milk. The extract, after evaporation of the solvent, is digested with sulfuric acid and perchloric acid, and the copper is determined colorimetrically by means of the carbamate method. They state that the accuracy of this procedure is unaffected by the presence of iron, tin, aluminum, lead, zinc, nickel, and manganese.

#### CONCLUSIONS

It is believed that with the experience gained this year in the collaborative study, further refinements can be made in the procedures for the elimination of interfering substances. A majority of the collaborators expressed a preference for the carbamate method, and since the Biazzo and carbamate procedures appear to have given comparable results in this year's study, it is recommended<sup>2</sup> that work be continued on methods employing sodium diethyldithiocarbamate and dithizone for determination of copper in foods.

#### REPORT ON FLUORINE IN FOODS\*

By DAN DAHLE (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The work done by the associate referee and H. J. Wichmann<sup>3</sup> on the separation of minute quantities of fluorine from aluminum salts indicated a possibility of using some suitable aluminum salt as a fixative for fluorine during the ashing of food products. Therefore experimental work was undertaken to study the conditions under which the presence of aluminum ions in excess would prevent the volatilization of small quantities of fluorine during the process of evaporation and incineration of organic matter.

Aluminum nitrate was chosen in preference to salts of aluminum with other volatile acids because (1) it is readily available, (2) it has great solu-

<sup>1</sup> *Analyst*, 60, 376 (1935).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

<sup>3</sup> This report is part of a thesis submitted to the American University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1936.

<sup>4</sup> *This Journal*, 19, 320 (1936)

bility, and (3) several batches were found containing less than 1 p.p.m. of fluorine.

Preliminary ashing experiments were conducted at 600° C. with fluorine-free mixtures containing varying concentrations of sugar, tartaric acid, and aluminum to which known amounts of fluorine were added as sodium fluoride. The ashes were distilled as suggested by Dahle and Wichmann<sup>1</sup> and fluorine was determined by the peroxidized titanium method.<sup>2</sup> The results are listed in Table 1.

TABLE 1.—*Effect of varying concentrations of reactants*

| Al<br>USED  | SUGAR<br>USED | TARTARIC<br>ACID USED | F (as NaF) |            | LOSS       |                 |
|-------------|---------------|-----------------------|------------|------------|------------|-----------------|
|             |               |                       | ADDED      | FOUND      |            |                 |
| <i>gram</i> | <i>grams</i>  | <i>grams</i>          | <i>mg.</i> | <i>mg.</i> | <i>mg.</i> | <i>per cent</i> |
| 0.3         | 5.0           | —                     | None       | None       | —          | —               |
| 0.3         | 5.0           | —                     | 0.5        | 0.462      | 0.038      | 7.6             |
| 0.6         | 5.0           | —                     | 0.5        | 0.500      | —          | —               |
| 0.6         | 5.0           | 1.0                   | 0.5        | 0.497      | 0.003      | 0.6             |
| 0.6         | 5.0           | 1.0                   | 2.0        | 1.640      | 0.360      | 18.0            |
| 0.6         | 5.0           | 1.0                   | 2.0        | 1.692      | 0.308      | 15.4            |
| 0.6         | 20.0          | 1.0                   | 0.5        | 0.504      | —          | —               |
| 0.6         | 20.0          | 1.0                   | 0.5        | 0.501      | —          | —               |
| 0.6         | 20.0          | 1.0                   | 0.5        | 0.505      | —          | —               |
| 0.6         | 40.0          | 1.0                   | 0.5        | 0.425      | 0.075      | 15.0            |
| 0.6         | 20.0          | 5.0                   | 0.5        | 0.475      | 0.025      | 5.0             |
| 0.6         | 20.0          | 1.0                   | 1.0        | 0.930      | 0.070      | 7.0             |
| 0.6         | 20.0          | 5.0                   | 2.0        | 1.480      | 0.520      | 26.0            |
| 0.6         | 20.0          | 5.0                   | 2.0        | 1.528      | 0.472      | 23.6            |
| 0.9         | 40.0          | 5.0                   | 2.0        | 1.944      | 0.056      | 2.8             |

From Table 1 it may be concluded—

(1) That in the presence of aluminum nitrate losses of fluorine can be avoided during ashing of organic matter even if the material be acidic in nature, *provided* the acidity is due to organic acids.

(2) That with a constant amount of aluminum nitrate an increase in (a) the total amount of organic solids, (b) the amount of acid, or (c) the amount of fluorine present will result in losses of fluorine.

(3) That such losses can again be prevented by a simultaneous increase in the amount of fixative.

Next the effect of ashing temperature was studied. As charges of 20 grams of sugar, 1 gram of tartaric acid, 0.5 mg. of fluorine as sodium fluoride and 0.6 gram of aluminum as nitrate had previously given complete recoveries when ashed at 600° C., they were again used in all cases except one, where 0.9 gram of aluminum was used as a fixative. Incinera-

<sup>1</sup> *This Journal*, 19, 327 (1936).

<sup>2</sup> *Ibid.*, 16, 612 (1933).

tions were made at 400°, 500°, 600°, 650°, and 700° C. The results are given in Table 2.

TABLE 2.—*Effect of varying ashing temperature*

| TEMPERATURE | F (as NaF) |       | LOSS  |          |
|-------------|------------|-------|-------|----------|
|             | ADDED      | FOUND |       |          |
| °C.         | mg.        | mg.   | mg.   | per cent |
| 400         | 0.5        | 0.503 | —     | —        |
| 400         | 0.5        | 0.499 | 0.001 | 0.2      |
| 500         | 0.5        | 0.501 | —     | —        |
| 500         | 0.5        | 0.489 | 0.011 | 2.2      |
| 500         | 0.5        | 0.480 | 0.020 | 4.0      |
| 600         | 0.5        | 0.502 | —     | —        |
| 600         | 0.5        | 0.497 | 0.003 | 0.6      |
| 600         | 0.5        | 0.498 | 0.002 | 0.4      |
| 650         | 0.5        | 0.477 | 0.023 | 4.6      |
| 700         | 0.5        | 0.347 | 0.153 | 30.6     |
| 700         | 0.5        | 0.347 | 0.153 | 30.6     |
| 700         | 0.5        | 0.314 | 0.186 | 37.2     |
| 700         | 0.5        | 0.304 | 0.196 | 39.2     |
| 700         | 0.5        | 0.355 | 0.145 | 29.0     |
| 700*        | 0.5        | 0.487 | 0.013 | 2.6      |

\* 0.9 g. of Al used as fixative.

From Table 2 it may be concluded—

(1) That complete ashing is not necessary if the subsequent fluorine determination is made by the peroxidized titanium method.

(2) That 600°–650° C. is a safe temperature to use in the incineration.

(3) That an increase in the amount of fixative results in increased recoveries, even above 650° C.

These preliminary experiments having indicated distinct possibilities for aluminum nitrate as a fluorine fixative during incineration, attention was next given to ashing of typical foods. The following products were tried: apples, lettuce, green tomatoes, flour, liver, and eggs.

The following procedure was followed:

A suitable aliquot of material was ashed at 600° C. in the presence of 0.6 gram of aluminum added as nitrate. The ashing was repeated on another aliquot, to which known amounts of sodium fluoride had been added. The ashes were distilled as described in a separate paper, p. 327, and fluorine was determined in the distillates.

Finally a distillation was made directly on the material itself, the distillate being evaporated in alkaline solution, oxidized, and redistilled. (This double distillation procedure, while somewhat limited in its scope of application, has been found to give accurate results if the peroxidized titanium method is being used for the determination of fluorine.)

Table 3 gives results by this procedure. The accuracy of the method is indicated by the fluorine content of the food product, calculated (1) from ashings with and without added known amounts of fluorine, and (2) from direct distillation.

These results, Table 3, indicate that the ashing with aluminum nitrate as a fluorine fixative is successful with a variety of products but not with all types.

TABLE 3.—*Food products ashed with Al(NO<sub>3</sub>)<sub>3</sub>*

| FOOD<br>PRODUCT | QUANTITY<br>USED | PROCEDURE<br>USED | F (as NaF) |            | DIFFERENCE | F CONTENT<br>INDICATED |
|-----------------|------------------|-------------------|------------|------------|------------|------------------------|
|                 |                  |                   | ADDED      | FOUND      |            |                        |
|                 | <i>grams</i>     |                   | <i>mg.</i> | <i>mg.</i> | <i>mg.</i> | <i>p.p.m.</i>          |
| Apples          | 200              | Al-Ash            | None       | 0.220      | 0.220      | 1.10                   |
| Apples          | 100              | Al-Ash            | 0.35       | 0.442      | 0.092      | 0.92                   |
| Apples          | 100              | Al-Ash            | 0.35       | 0.465      | 0.115      | 1.15                   |
| Apples          | 100              | Al-Ash            | 0.35       | 0.482      | 0.132      | 1.32                   |
| Apples          | 50               | Direct Dist.      | None       | 0.065      | 0.065      | 1.30                   |
| Lettuce         | 100              | Al-Ash            | None       | 0.079      | 0.079      | 0.79                   |
| Lettuce         | 100              | Al-Ash            | 0.50       | 0.555      | 0.055      | 0.55                   |
| Lettuce         | 100              | Al-Ash            | 0.50       | 0.556      | 0.056      | 0.56                   |
| Lettuce         | 100              | Al-Ash            | 0.50       | 0.563      | 0.063      | 0.63                   |
| Lettuce         | 70               | Direct Dist.      | None       | 0.050      | 0.050      | 0.71                   |
| Tomatoes        | 70               | Direct Dist.      | None       | 0.050      | 0.050      | 0.71                   |
| Tomatoes        | 70               | Direct Dist.      | None       | 0.050      | 0.050      | 0.71                   |
| Tomatoes        | 70               | Al-Ash            | None       | 0.060      | 0.060      | 0.86                   |
| Tomatoes        | 70               | Al-Ash            | 0.40       | 0.445      | 0.045      | 0.64                   |
| Tomatoes        | 70               | Al-Ash            | 0.80       | 0.857      | 0.057      | 0.81                   |
| Flour           | 20               | Al-Ash            | None       | 0.021      | 0.021      | 1.05                   |
| Flour           | 20               | Al-Ash            | 0.40       | 0.423      | 0.023      | 1.15                   |
| Liver           | 70               | Al-Ash            | None       | 0.100      | 0.100      | 1.43                   |
| Liver           | 70               | Al-Ash            | 0.40       | 0.511      | 0.111      | 1.59                   |
| Liver           | 25               | Direct Dist.      | None       | 0.038      | 0.038      | 1.52                   |
| Egg White       | 19               | Direct Dist.      | None       | 0.009      | 0.009      | 0.47                   |
| Egg White       | 57               | Direct Dist.      | None       | 0.018      | 0.018      | 0.32                   |
| Egg White       | 32               | Al-Ash            | None       | 0.0044     | 0.0044     | 0.14                   |
| Egg White       | 90               | Al-Ash            | 0.045      | 0.030      | -0.105     | None                   |
| Egg Yolk        | 20               | Direct Dist.      | None       | 0.024      | 0.024      | 1.20                   |
| Egg Yolk        | 54               | Al-Ash            | None       | 0.0227     | 0.0227     | 0.42                   |

With eggs, both white and yolk, the results by ashing were distinctly lower than those found by direct distillation, and when known quantities of sodium fluoride were added, not even the full amount added was recovered. While these low results may be due in part to withdrawal of aluminum by the  $P_2O_5$  in the egg, with formation of insoluble aluminum phosphate, the losses would seem to be chiefly attributable to lack of intimate contact between sample and fixative. Although the aluminum nitrate solution was added slowly and with constant stirring, coagulation could not be prevented. This resulted in a lumpy mixture, only the surface of which was in contact with the fixative. This problem of intimate contact would in all probability apply to other fixatives as well. With eggs, a preliminary coagulation at as low a temperature as possible, fol-

lowed by a thorough mechanical disintegration, should evidently precede the addition of the fixative.

In its present state the method of ashing with aluminum nitrate is slow. The separation of fluorine from the aluminum in the ash as reported elsewhere<sup>1</sup> is exacting. Ashing with lime, as reported last year by the referee, has many advantages, and it may gain in favor as limes low in fluorine are now on the market.

#### RECOMMENDATIONS<sup>2</sup>

It is recommended—

- (1) That the work on fluorine in foods be continued.
- (2) That a comparison of the relative merits of lime ashing and aluminum ashing be made.
- (3) That the evaluation of existing methods of determination be again studied.

#### REPORT ON LEAD

By P. A. CLIFFORD (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The associate referee devoted the past year to a study of dithizone methods for lead. The colorimetric method, announced two years ago for the determination of lead as spray residue on fruits, was modified to make it of general application and was refined in order to determine accurately smaller amounts of lead than has heretofore been found possible. The mechanism of the dithizone reaction was studied thoroughly, and instruments for measuring the color were developed for the purpose of increasing accuracy and speed. This work is summarized in a contributed paper appearing in *This Journal*, 19, 130 (1936).

The associate referee recommends<sup>2</sup> (1) simplification of the methods for the removal of bismuth and tin interference; (2) further work on products such as oils and baking powders, which may require special preliminary treatment; (3) development of proper methods of sampling for products such as canned sardines; (4) adoption of the electrolytic and colorimetric dithizone procedures for the determination of lead as alternative tentative methods; and (5) collaborative work with the view to making these methods official.

<sup>1</sup> Dahle and Wichmann, *This Journal*, 19, 327 (1936).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

## REPORT ON MERCURY

By W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

During the year several methods for the determination of minute quantities of mercury were investigated. Among these were the electrometric method of Bodnor and Szep,<sup>1</sup> the potentiometric method of Schwarz and Kantor,<sup>2</sup> and the dithizone method proposed by the associate referee.

The electrometric method involves the plating out of the mercury upon iron wire, followed by its distillation and measurement as a globule. In this method difficulty was encountered in reducing the amount of mercury solution to about 1 cc. of volume, while in the distillation it appeared that small amounts of mercury adhered to the glass tube and could not be completely distilled. The distillate was often contaminated by what appeared to be copper oxide. A small amount of copper solution was used to aid in the deposition of the mercury on the iron wire. The technic of this determination requires considerable practice and skill, and the method, in the writer's opinion, is not suitable for regulatory work.

The potentiometric method has some advantages over the electrometric, but with the apparatus described the sensitivity is not sufficient for accurate work. The apparatus possesses too much internal resistance. The method has not been sufficiently investigated to form a definite conclusion as to its merits for regulatory work.

A dithizone method was formulated by the associate referee and was published in *This Journal*, 18, 638 (1935). It has been further studied this year as described below.

## INCREASING THE ACCURACY OF THE DITHIZONE METHOD

The limit of accuracy of the method as published is about 3.5 gamma (0.003 to 0.005 mg.), within a range of 0–0.5 mg. Attempts were made to increase this accuracy to 1 gamma. The chief difficulty encountered was the fading of the dithizone when present in such small quantities. The dithizone in the preliminary or first extractions used to isolate the mercury is oxidized with acid permanganate solution. This oxidizing agent is preferred to all the others tested, including ammonium persulfate, sodium bromate, nitric and sulfuric acids, perchloric and sulfuric acids, and potassium dichromate.

Reduction of the excess permanganate presented considerable difficulty. Hydrogen peroxide, hydrazine sulfate, oxalic acid, sodium sulfite, hydroxylamine, sodium arsenite, and other reducing agents were tried. With each of these reducing agents some fading of the dithizone resulted,

<sup>1</sup> *Biochem. Z.*, 205, 219 (1929).

<sup>2</sup> *Mikrochemie*, 13, 225 (1933).



although in the case of sodium arsenite it was slight. The reaction, however, in the presence of the arsenite is 'quite sluggish. Reduction with hydrogen peroxide has the disadvantage that the oxygen liberated in the reaction is difficult to remove and causes some fading of the dithizone reagent.

The use of nitrites as the reducing agent was purposely avoided because of their destructive action upon dithizone. The associate referee has recently found that hydroxylamine salts will destroy nitrites completely at 60° C. If acid permanganate solutions are reduced with nitrites followed by the decomposition of nitrites with hydroxylamine sulfate or chloride, the solution can be titrated without fading of the dithizone. The accuracy can therefore be increased to within 1 gamma within the limits previously given. It is desirable to incorporate this change in the published procedure. The titration as published can be simplified by adding a measured quantity (1 cc. = .01 mg.) of standard mercury at the end of the titration when the end point is slightly past and then titrating the excess and correcting for the amount added.

Formerly, the dithizone extract containing the mercury was evaporated to dryness and the oxidation was performed on the steam bath with acid permanganate. This procedure can be followed at the option of the analyst although the direct oxidation saves time.

#### DIRECT EXTRACTION OF MERCURY

Since the oxidation of the sample solution requires more time than any other part of the procedure, methods of extracting the mercury directly from the acid extract without oxidation were tried, but without complete success. The destruction of the dithizone by the nitrites that were present at first prevented the direct extraction of mercury from the nitric acid solution, but it was found that sufficient hydroxylamine sulfate or hydrochloride will destroy nitrites as fast as formed and prevent disintegration of the dithizone. The use of hydroxylamine salts for this purpose, however, did not solve the difficulty.

Several samples of lettuce to which were added known quantities of mercury were extracted without complete recovery of the mercury. Extraction of the mercury directly from the nitric acid extract of the lettuce after the solution had been made ammoniacal, did not give complete recovery, especially in the presence of interfering substances, when only about 50 per cent was recovered. The formation of free mercury in the ammoniacal solution, especially in the presence of chlorides, is probably responsible for this fact.

#### MERCURY IN LETTUCE

To determine the natural content of mercury in lettuce, a number of samples of undusted lettuce were secured and analyzed by the permanganate oxidation method. Results indicated little or no mercury. The

blank of 15 gamma on the reagents was much too high to permit an accurate determination of the mercury content of the lettuce. Seven gamma was present in the nitric acid used and the rest in the permanganate or hydrogen peroxide, or both.

Samples of lettuce to which were added known quantities of mercury together with interfering substances were submitted to two collaborators. One collaborator recovered 0.143 mg. when 0.143 mg. was present and the other recovered .073 mg. when .075 mg. was present. On many trials of the method the associate referee recovered to within .003 to .005 mg. of the amount present. With the modification indicated above, an accuracy of one gamma should be obtained.

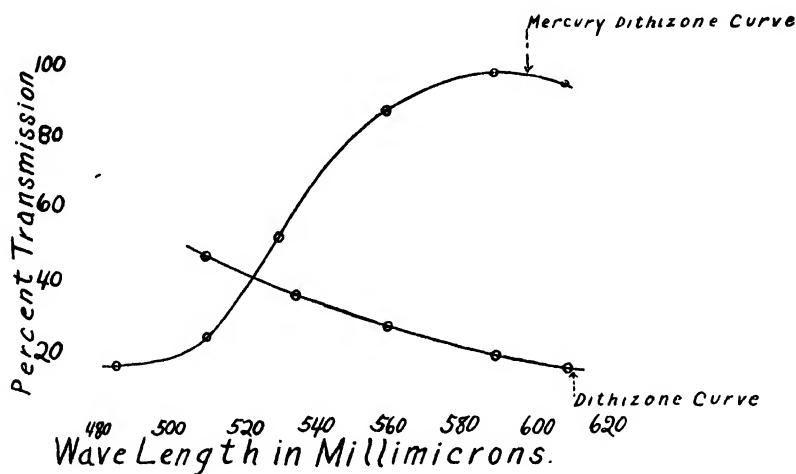


FIG. 1

It is possible that further study of the underlying principles would permit shortening the procedure to some extent without impairing its accuracy. The optical methods used by P. A. Clifford<sup>1</sup> for the determination of traces of lead might be applicable to mercury. Some preliminary work on the transmission of light of various wave lengths through dithizone solutions and mercury-dithizone solutions indicates a wide spread in the transmissions at a wave length of about 600 millimicrons. The curve of transmission at various wave lengths is given in Fig. 1. An examination of the curves indicates that the use of the two color system as applied to lead can be applied to mercury also.

#### RECOMMENDATIONS<sup>2</sup>

It is recommended—

- (1) That the dithizone method published in *This Journal*, 18, 639 (1935) be modified as directed below and be adopted as tentative.

<sup>1</sup> *This Journal*, 19, 130 (1936).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

Change the sentence (line 15, p. 641), "(If particles of precipitate are hard to dissolve, heating may facilitate solution)" to read "Replace the condenser and heat to boiling 5 minutes to remove free oxygen and to dissolve refractory particles, then cool again."

Omit the sentence beginning "First make a preliminary or rough titration . . ." (line 4 from bottom of p. 641).

Change par. 6, p. 641, beginning "Add to the combined extracts . . .," to read "Oxidize the combined dithizone extracts by either of the following methods:

(1) Warm the oxidizing mixture composed of 50 cc. of water, 10 cc. of 5%  $\text{KMnO}_4$  solution and 2 cc. of  $\text{H}_2\text{SO}_4$  (1+1) to 50–55° C. and add to the extracts in a separatory funnel. Shake gently at first, release pressure, then shake vigorously for several minutes.

(2) Evaporate the extracts to dryness on the steam bath. Add the oxidizing mixture described in (1) and allow to remain on the steam bath for 15–20 minutes.

Add sufficient 10%  $\text{NaNO}_2$  solution dropwise to clear the solution obtained under (1) or (2). Shake or stir after the addition of a few drops. To the clear solution add 0.75 gram of hydroxylamine sulfate or chloride. Add permanganate to the standard and clear it in the same manner before making the titration."

Change lines 11 to 27 (p. 642), beginning "The change of color is the end point . . .," to read "Add sufficient standard mercury solution (usually 1 cc. = .01 mg.) to the titrated sample to supply an excess of mercury, shake, and draw off the extract. Titrate the excess of mercury with the dithizone solution, adding a few tenths cc. of the solution at a time. Subtract the mercury added. Titrate the standard in the same manner and calculate the mercury in the sample."

(2) That the study of methods for mercury be continued.

## REPORT ON SELENIUM

By R. A. OSBORN (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The interest shown recently in methods for the quantitative determination of small amounts of selenium in organic material and in soils is occasioned by the discovery of widespread natural deposits of this element in soils throughout the world, and by the fact that selenium in small quantities is toxic to both plant and animal life.

It is desirable to discuss recent analytical studies on selenium under the headings: (1) Preparation of the sample for analysis, (2) isolation of selenium from interferences, and (3) determination. Some analysts use a wet digestion procedure for sample preparation while others prefer an alkaline ashing. W. O. Robinson<sup>1</sup> and A. K. Klein,<sup>2</sup> working independently, have used an alkaline ashing procedure with success. There is also a difference of opinion in regard to measuring the selenium, which subject will be discussed later. All workers in this field seem to consider that distillation of selenium from constant boiling hydrobromic acid constitutes a satisfactory method of isolation.

<sup>1</sup> Unpublished.

<sup>2</sup> Unpublished

The methods most commonly used by workers in the Department of Agriculture for the determination of selenium are those described by W. O. Robinson and others.<sup>1</sup> Samples of organic material are oxidized with concentrated sulfuric acid in an all-glass combination digestion-distillation apparatus with provision for scrubbing the volatile products of the oxidation. A mixture of concentrated hydrobromic acid and free bromine is then added to the digest, and selenium is distilled as a volatile bromide. It is then precipitated by the addition of sulfur dioxide and hydroxylamine hydrochloride and either weighed or measured turbidimetrically.

More recently Williams and Lakin<sup>2</sup> published a modification of this procedure for the determination of selenium in organic material, which simplifies the digestion of the sample. Samples are digested in open beakers with concentrated nitric and sulfuric acids. Apparently, this modification works well provided proper consideration is given to such factors as temperature and time. The difficulty of testing this, or any other procedure, lies in the chemist's inability to select as a standard an organic substance similar to a plant material containing a known amount of selenium. Complete recoveries of added inorganic selenium by a given procedure do not demonstrate that selenium in organic form will likewise be quantitatively recovered. To date the method giving the highest results is considered the most accurate. In view of the known ease of reduction of selenium and its volatility at high temperatures when in the reduced state, it is possible that existing procedures may be giving low results.

The modification of Greathouse<sup>3</sup> involves a change in the method of sample preparation. The sample is subjected to a preliminary digestion with dilute sodium hydroxide solution, followed by treatment with dilute sulfuric acid and sodium bromate, which oxidizes the organic material. The digestion is carried out in an all-glass apparatus similar to that described by Robinson et al.<sup>4</sup>

The Shorey method,<sup>5</sup> likewise, differs only in the type of digestion procedure. The organic sample is refluxed with an excess of free bromine in an all-glass system, and no attempt is made to destroy the organic material. After 30–45 minutes of refluxing, concentrated hydrobromic acid is added and the selenium is distilled.

The associate referee studied the effect of the addition of ferrous sulfate to the sample during digestion. The sample was digested with concentrated sulfuric acid to which was added 10 grams of solid ferrous sulfate in an all-glass apparatus similar to Robinson's but no attempt was made to scrub the gases evolved.

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<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 6, 274 (1934).

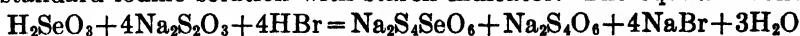
<sup>2</sup> *Ibid.*, 7, 409 (1935).

<sup>3</sup> Unpublished.

<sup>4</sup> *Loc. cit.*

<sup>5</sup> Unpublished.

Klein<sup>1</sup> uses a volumetric method after distillation from the bromine-hydrobromic acid solution. Phenol solution is added to destroy free bromine, and the selenious acid is determined by the addition of an excess of standard sodium thiosulfate, the excess thiosulfate being titrated with a standard iodine solution with starch indicator.<sup>2</sup> The equation follows:



In another volumetric method described by Hillebrand and Lundell<sup>3</sup> the selenious acid is reduced with hydriodic acid with the formation of free iodine, which is titrated with standard thiosulfate with starch as an indicator. Equations for the reactions follow:  $\text{H}_2\text{SeO}_3 + 4\text{HI} = 4\text{I} + \text{Se} + 3\text{H}_2\text{O}$ ;  $4\text{I} + 4\text{Na}_2\text{S}_2\text{O}_3 = 2\text{Na}_2\text{S}_4\text{O}_6 + 4\text{NaI}$ .

Collaborator Klein reports that he secured fairly satisfactory results by this method, but he expresses a preference for the first method because he finds it easier to approach the end point when selenium is not present as a precipitate. Both of these volumetric methods should be given critical consideration from a laboratory standpoint, as they may give better results than does the turbidimetric procedure. In Robinson's turbidimetric method the quantities of selenium ordinarily measured are .01 to .50 mg. H. L. Greathouse and A. L. Curl, working independently, made refinements in the turbidimetric procedure by eliminating gum acacia as a suspending agent, developing a more reproducible method of precipitation, and viewing the turbidity in a dark room by the Tyndall beam effect. As a result of these refinements they were able to detect a quantity of selenium as small as .002 mg. suspended in 25 cc. Since the presentation of this report, Curl has carefully studied the turbidimetric range and reproducibility within the range (unpublished work in the U. S. Food and Drug Administration). He reports turbidimetric measurements from 0.002 to 2.000 mg. of selenium with recoveries within 15 to 20 per cent of the amount added. If greater accuracy than this is desired, it would appear to be better to develop volumetric or colorimetric methods, which in general are more accurate, than to attempt to improve the turbidimetric procedure.

The gravimetric procedure appears to be reasonably satisfactory with quantities of 1.0 mg. or more. The analyst should have available a good balance and small fritted glass Gooch crucibles on which to filter, wash, dry, and weigh the selenium.

Horn<sup>4</sup> describes a qualitative colorimetric test for selenium in organic compounds, using codeine sulfate, and suggests that the test has quantitative possibilities. J. Davidson of the U. S. Bureau of Chemistry and Soils has recently made a study (unpublished) of the quantitative aspects of the use of this reagent, and he states that he is able to measure selenium quantitatively. This possibility should receive consideration.

<sup>1</sup> Unpublished

<sup>2</sup> Norris and Fay, *Am. Chem. J.*, 23, 119 (1900).

<sup>3</sup> Applied Inorganic Analysis, p. 266 (1929).

<sup>4</sup> *Ind. Eng. Chem. Anal. Ed.*, 6, 34 (1934).

Table 1 indicates the quantities of selenium found by analysts of the U. S. Department of Agriculture, each analyzing two or more of three standard samples: a dry vegetation, high in selenium (approximately 1200 p.p.m.), a dry vegetation relatively low in selenium (5-15 p.p.m.), and a soil (5-10 p.p.m.).

TABLE 1.—*Collaborative results of selenium determinations*

| ANALYST | DRY VEGETATION<br>SAMPLE 7                      | DRY VEGETATION<br>SAMPLE 8              | SOIL<br>SAMPLE 9  |
|---------|---|---|-------------------|
|         | p.p.m.  | p.p.m.                                  | p.p.m.            |
| G       | 1160, 1170 (1)<br>1195, 1225 (3)                | 12, 14 (1)<br>15, 17 (3)                |                   |
| K       | 1200, 1300 (1)<br>1174, 1180 (5)*               | 8.6 (5)                                 | 5 (1)<br>5.2 (5)* |
| L       | 1150 1180 (1)<br>1260 1280 (2)                  | 8, 10, 12, 15 (1)<br>12, 14, 15, 15 (4) | 9, 10 (1)         |
| O       | 1026 1170 (1)<br>1146 1236 (2)<br>1040 1040 (6) | 5, 6, 5 (1)                             |                   |
| S       | 1170 1200 (4)                                   | 14, 15 (4)                              |                   |
| W       | 1215 1230 (1)                                   | 5, 8, 12 (1)                            | 10, 10 (1)        |

\* Sample ashed.

(1) Procedure described by Robinson et al.

(2) Procedure described by Williams and Lakin.

(3) Unpublished procedure of H. L. Greathouse.

(4) Unpublished procedure of E. C. Shorey.

(5) Unpublished procedure of A. K. Klein.

(6) Unpublished procedure of R. A. Osborn.

In considering the results presented, it should be borne in mind that the unpublished procedures may be further improved by additional studies. Then, too, the data in the table are inadequate for the purpose of drawing final conclusions as to the relative merits of the various modifications. It appears, however, that somewhat higher results are obtained by Procedures (2) and (3) than by Procedure (1). It would appear that the variations in the results by the several procedures and within individual procedures are in several instances too large to be explained by non-uniformity of the samples. It is more probable that the lower values are due to loss of selenium at some stage of the analysis. Losses are possible during sample digestion (1) when large volumes of gaseous products are evolved and a high temperature is maintained for a considerable length of time; (2) during precipitation of selenium with sulfur dioxide gas (if or when a large excess of free bromine has been distilled, which could produce a marked elevation of the temperature of the solution, and would cause the formation of an unusually high concentration of hydro-

bromic and sulfuric acids, under which condition the loss could occur either by distillation of selenium as a volatile bromide or by its incomplete precipitation in the more highly acid solution); or (3) during digestion of the precipitate (if the temperature and digestion time are not carefully controlled, and the sulfur dioxide is lost by volatilization or oxidation, under which conditions selenium may redissolve and slowly volatilize).

It is recommended<sup>1</sup> that study of methods for the determination of selenium be continued and that particular attention be given to methods of sample preparation and determination.

## REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

With the exception of minor changes, the revision of the existing procedures in the chapter on Fruits and Fruit Products required little attention. The changes in the methods authorized by the Association have been introduced, and new methods, principally on organic acids, have been added. The procedure for active (levo) malic acid was adopted tentatively at the last meeting. The procedure for the inactive acid has not been subjected to collaborative study. However, because of satisfactory application in official work, its inclusion in the chapter seems justified. The method for the determination of lactic acid described at the conclusion of this report has not been studied in its application to fruit products by the Association, so that its introduction in the chapter is not suggested at this time, although the satisfactory collaborative data on wines would seem to justify such action, particularly since wines contain many products of fermentation not occurring in fruit juices. The text of the Kling method for tartaric acid described in *This Journal*, 13, 109 (1930), has been substituted for that of the present method.

No reports were submitted by the Associate Referees on Soluble Solids and Effect of Acids on Sugars during Drying and on Pectic Acid and Electrometric Titration.

During the year a method for lactic acid was developed by the referee. In principle it is an adaptation of the procedure published by Moeslinger<sup>2</sup> about 35 years ago, in which the acid is isolated as the barium salt with 80 per cent alcohol and calculated from the alkalinity of the ash.

### PROPOSED METHOD FOR LACTIC ACID IN FRUITS AND FRUIT PRODUCTS

Evaporate 200 cc. of a sample solution of a jam or jelly, 1(b),<sup>3</sup> to about 50 cc. and transfer to a 250 cc. volumetric flask with about 75 cc. of H<sub>2</sub>O. To the solution

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

<sup>2</sup> *Z. Untersuch. Nahr. Genussm.*, 4, 1120 (1901).

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1930, 264.

add 0.2 A gram of powdered  $\text{Ba}(\text{OH})_2 \cdot 8 \text{H}_2\text{O}$ , in which "A" is the titratable acidity of the 200 cc. sample solution in terms of normal acid. Shake vigorously for a few minutes and heat to  $60^\circ$ . Immediately, while still warm, add 50 cc. 95% alcohol and shake 2 minutes. Add a second portion of 50 cc. alcohol and again shake 2 minutes. Add alcohol to mark and shake 2 minutes. Cool to  $20^\circ$ , adjust to mark with alcohol, mix, and allow to stand 5 minutes, shaking at frequent intervals. Filter through a large folded paper (drain thoroughly), add 7 drops of  $\text{H}_2\text{SO}_4$  (1+1) to the filtrate and mix. Concentrate by aspiration 200 cc. of the acidified filtrate in the following manner: Fit a long-necked round-bottomed flask of 300 cc. capacity with a rubber stopper carrying 2 glass tubes, one extending just through the stopper and the other passing to the bottom of the flask. The latter tube is perforated at its lower end to produce a spraying effect. Pipet 100 cc. of the alcoholic solution into the 300 cc. flask, insert the sprayer, apply gentle suction to the short tube, and immerse in a briskly boiling water bath. Adjust to the greatest suction the conditions will permit and concentrate to about 50 cc. If during aspiration there is the tendency to foam, add a few more drops of  $\text{H}_2\text{SO}_4$  (1+1). Now pipet a second 100 cc. portion of the solution into the flask and reduce to a volume of about 30 cc. Add 25 cc. of  $\text{H}_2\text{O}$  and again reduce to about 30 cc. Rinse with  $\text{H}_2\text{O}$  into a 50 cc. volumetric flask, cool to  $20^\circ$ , add 1 cc. of  $\text{H}_2\text{SO}_4$  (1+1) and dilute with  $\text{H}_2\text{O}$  to mark. Shake, and filter through a hardened paper, pouring back until *clear*. Pipet 25 cc. into the extractor described in *This Journal*, 16, 439 (1933) and extract 3 hours with washed ether. To the ether extract add 15 cc. of  $\text{H}_2\text{O}$ , stopper the flask, and shake vigorously. Expel the ether on the steam bath, rinse into the aspirator flask with about 35 cc. of  $\text{H}_2\text{O}$ , insert aspirator, immerse in a briskly boiling glycerol bath (30%), and steam distil until a 250 cc. distillate has been collected. Collect an additional 20 cc. distillate and test neutrality with 0.1 N alkali, using phenolphthalein indicator. Generally 1 drop of alkali will produce a decided pink color. If this is not the case, continue the distillation until 20 cc. gives a decided pink with 1 drop of alkali. Rinse the contents of the aspirator flask into an evaporating dish (platinum preferred) and evaporate to about 50 cc. Carefully neutralize with baryta water (phenolphthalein) and evaporate to about 15 cc. Adjust the weight of solution in the dish to 20 grams (nearest gram) and rinse with 95% alcohol into a 100-110 cc. volumetric flask. Dilute to 110 cc. with alcohol, shake, allow to stand 10 minutes, and filter through a folded paper, pouring back until *bright*. Evaporate 100 cc. of the alcoholic filtrate to dryness, ash, and determine the alkalinity of the ash, using phenolphthalein indicator. 1 cc. of 0.1 normal acid = 0.009 gram of lactic acid.

The ash need not be white. Heat to dull redness, add a small quantity of  $\text{H}_2\text{O}$ , heat to boiling, evaporate to dryness, and again heat to dull redness. Add about 10 cc. of  $\text{H}_2\text{O}$ , heat to boiling, add an excess of 0.1 N HCl (20 cc.), boil, and titrate back with 0.1 N alkali.

#### EXPERIMENTAL

The procedure was applied to a preparation composed of the various constituents (except lactic acid) ordinarily occurring in a wine of the fortified type. Four determinations indicated 13, 13, 15, and 15 mg. of lactic acid per 100 grams, or about 0.8 mg. in the aliquot. It may be assumed that these small quantities of apparent lactic acid are in the main attributable to acetic acid, which is not entirely volatilized in steam distillation. The distillate from a solution containing 5 cc. of 0.1 N acetic acid, the quantity ordinarily present at this step in the examination of a wine, showed an acidity of 4.80 cc. Similar experiments in which a few



drops of sirupy lactic acid were added to the acetic acid solution showed titrations of 4.95 cc. It is apparent from these experiments that lactic acid is slightly volatile under the conditions prescribed for the steam distillation. Since the acetic acid remaining in the distilling flask practically compensates the loss of lactic acid through volatilization, a correction at this point of the procedure is not required.

Six determinations on a mixture of 100 grams of the synthetic wine and 374 mg. of lactic acid showed 365, 361, 363, 368, 370, and 362 mg., a recovery of 97.5 per cent. An apple jelly prepared in the laboratory showed 25, 23, and 25 mg. of lactic acid per 100 grams. After the addition of 156 mg. of the acid the mixture yielded 175, 173, 173, and 173 mg., a recovery of 96.4 per cent.

Collaborative results reported by three chemists of the Food Division, U. S. Food and Drug Administration, Washington, D. C., on a commercial port wine showed (a) 220, 220; (b) 230, 240; (c) 220 and 220 mg. of lactic acid per 100 grams of wine.

From the data here presented it is believed that the method described is accurate, and it is recommended that it be subjected to collaborative study.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the method for the determination of inactive malic acid described in *This Journal*, 16, 281 (1933), be adopted as tentative.

(2) That the text of the Kling tartaric acid method described in *This Journal*, 13, 109 (1930) be substituted for that of the present Kling method.

(3) That the method for the determination of lactic acid described in this report be studied collaboratively.

(4) That the study on soluble solids and effect of acids on sugars on drying be continued.

(5) That the study on pectic acid and electrometric titration be continued.

No report on soluble solids and effect of acids on sugar on drying was given by the associate referee.

No report on pectic acid and electrolytic titration acidity was given by the associate referee.

For report on fruit acids see report of the Referee on Fruits and Fruit Products.

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 59 (1936).

## REPORT ON VITAMINS

By E. M. NELSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Pursuant to the recommendations made by the Referee on Vitamins last year, studies were conducted on the following problems: The application of spectrophotometric methods to the determination of vitamin A in fish and fish liver oils, the determination of vitamin D in milk, the determination of vitamin B<sub>1</sub> in feeding stuffs, the technic and details of the determination of vitamin D using chicks as the test animals, and the determination of vitamin D by means of chicks in feeding stuffs with vitamin D added. These last two problems, which relate directly to the tentative method for vitamin D assay by the preventive biological method, together with the method for vitamin B, are a continuation of studies initiated under the Referee on Feeding Stuffs. All of these studies show important progress in the development of information relative to the respective methods involved, and the referee wishes to take this opportunity of commending the work of the associate referees. It is gratifying indeed to have competent investigators devote a portion of their time to the development of assay methods for vitamins. No recommendations for the adoption of methods were made by the associate referees, and all of the recommendations for continued study meet with the referee's approval.

The development and adoption of official methods for the determination of vitamins B<sub>1</sub> and C, which are generally applicable, are most important. The work now being done on vitamin B<sub>1</sub> relates particularly to the determination of that vitamin in poultry feeds and is intended to serve as a foundation for the determination of other necessary components of the vitamin B complex for poultry, which appeared to be of very definite practical importance. More than a year ago the U. S. Pharmacopocia Revision Committee initiated an elaborate study on methods for the determination of vitamin B<sub>1</sub> in connection with a consideration of the inclusion of dried yeast in the U. S. Pharmacopocia. Therefore the referee considered that it was not important to appoint an associate referee for the determination of vitamin B<sub>1</sub> until the results of the collaborative studies now being sponsored by the Chairman of the U.S.P. Revision Committee were made known, and as a member of the U.S.P. Vitamin Advisory Board he is familiar with the progress of those studies since the results obtained are sent to him for review.

The International Standard for vitamin C did not become available until June of this year. Inasmuch as that was rather late for initiating any studies relating to that vitamin, no recommendations were made for the Associate Referee on Vitamin C. The referee should like to consider recommendations for investigators of methods for this vitamin.

At an informal meeting held a year ago and attended by eleven feed control officials and representatives of the industry, the Referee on Vitamins discussed the desirability of making available a cod liver oil of known vitamin D potency to serve as a standard for vitamin D determinations with chicks. The proposal made seemed to meet with the approval of all present. The purpose of such a standard is to eliminate difficulties in the interpretation of data obtained on biological methods in cases where the response of the animal must be used entirely in evaluating the results. This standard cod liver oil is intended to serve in the same manner for vitamin D assay with chicks as U.S.P. reference cod liver oil now serves in the U.S.P. official method for determination of vitamin D. It was suggested that this oil could be obtained and distributed through some U. S. Department of Agriculture laboratory, with the Association of Official Agricultural Chemists providing the funds necessary for purchase of the oil and suitable containers for its distribution. The price charged for the oil issued to analysts should be sufficient to cover the original outlay by the A.O.A.C. It has been ascertained that no objection will be offered to the preparation and distribution of such an oil by the Chairman of the U.S.P. Revision Committee, because of possible confusion with the U.S.P. reference cod liver oil.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended that a standard cod liver oil for vitamin D assays be made available as indicated. Such a program will entail (1) securing funds from the A.O.A.C. for the purchase of at least two barrels of cod liver oil\* and small containers for the distribution of samples; (2) securing a laboratory that will package and distribute the oil; and (3) appointment of an associate referee to supervise collaborative assays of the oil to establish its vitamin D potency and to be responsible for carrying out the program after the facilities have been secured.

### REPORT ON VITAMIN A

#### DETERMINATIONS WITH THE HILGER VITAMETER

By F. W. IRISH (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

For a number of years investigators, prominent among them Morton and Heilbron, have been using spectroscopic methods to study the spectral absorption curve of potent preparations of vitamin A made from various fish oils. The most important characteristic of this absorption

<sup>1</sup> For report of Subcommittee and action of the Association, see *This Journal*, 19, 52 (1936).

\* Several cod liver oil producers have indicated a willingness to donate cod liver oil for this purpose.

curve is the pronounced maximum in the region of 3280 Ångstrom units, and it has been found that the amount of absorption of ultraviolet light of this wave length can be used to measure the potency of vitamin A concentrates and, under properly-controlled conditions, of fish oils.

The general adoption of this method for determining vitamin A has been hampered by the fact that the equipment generally used for the purpose, i.e., a spectrophotometer, is quite expensive and the technic highly specialized. However, a few years ago a modified form of spectrophotometer was placed on the market by one of the well-known manufacturers of precision optical instruments.

This instrument, the Vitameter, is designed to measure the amount of absorption of ultraviolet light of wave length of 3280 Ångstrom units by means of an optical system which permits only light of approximately this wave length to pass through the instrument. Half of this beam of ultraviolet light passes through a cell containing a solution of the material under examination, and the other half passes through an aperture whose size is controlled by a shutter arrangement so that the size of the opening can be read on a graduated scale. The two beams then strike a fluorescent screen, producing two parallel green lines which are matched in intensity, and the setting of the shutter is read from the scale.

The distributors of the Vitameter provide certain instructions for the preparation of the solution of the oil being tested, and also a formula to be used in calculating the vitamin A potency of the oil from the scale reading. This instrument was welcomed by investigators in the field in the hope that it would enable them to dispense to a large extent with the laborious and expensive biological tests employed to determine the vitamin A content of fish oils, and other materials. However, it was soon found by several investigators<sup>1</sup> that the procedure and calculations proposed by the instrument-maker did not give results which harmonized with those obtained by means of biological tests, nor were the results in agreement with the established potency of the U.S.P. reference cod liver oil.

Various proposals have been made as to how the procedure and calculations should be varied to produce concordant results, and during the past year the associate referee has carried on a preliminary collaborative study in an attempt to gather information which it was hoped would prove useful in formulating a rapid and satisfactory method for the determination of vitamin A in fish oils.

Five samples of cod liver oils of known vitamin A potency were sent to eleven collaborators, who were requested to make vitamin A determinations with the Vitameter by means of three procedures:

(1) Strict compliance with the manipulations and calculations recommended by the manufacturers of the instrument.

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<sup>1</sup> Personal communications.

(2) The use of whatever modification of the procedure the collaborator considers most satisfactory.

(3) Calibration of the Vitameter against U.S.P. reference cod liver oil and calculation of the potency of the samples by means of this calibration.

The eight collaborators who reported on procedure No. 1 obtained the following results (Table 1):

TABLE 1.—*Results obtained by collaborators using instrument-maker's procedure*

| COLLABORATOR        | SAMPLE 1 | SAMPLE 2                                 | SAMPLE 3                 | SAMPLE 4                 | SAMPLE 5  |
|---------------------|----------|--|--------------------------|--------------------------|---|
| 1                   | 640      | 2080                                     | 960                      | 1270                     | 980   |
| 3                   | 773      | 2205                                     | 1074                     | 1524                     | 1035  |
| 4                   | 759      | 2270                                     | 1161                     | 1534                     | 1246  |
| 5                   | 638      | 2488                                     | 957                      | 1408                     | 968   |
| 6                   | 360      | 1731                                     | 712                      | 901                      | 533   |
| 8                   | 690      | 2462                                     | 1153                     | 1790                     | 1171  |
| 10                  | 572      | 1914                                     | 860                      | 1228                     | 830   |
| 11                  | 816      | 2928                                     | 1152                     | 1632                     | 1296  |
| Average             | 656      | 2259                                     | 1003                     | 1410                     | 1007  |
| Biological<br>Value | 750      | (U.S.P. Ref-<br>erence Cod<br>Liver Oil) | (78% No. 1<br>22% No. 2) | (55% No. 1<br>45% No. 2) | (Composite<br>of 21 com'l<br>cod liver<br>oils) |

The deviation from the average result for each oil is considerable, amounting in one case to as much as 47 per cent.

Of the collaborators who reported results obtained by the method which was in their opinion most satisfactory, six made the determination on the untreated oil, and five on the unsaponifiable fraction. The results from each of these two general methods of procedure are shown as (a) and (b) in Table 2.

The results reported by the eight collaborators who calibrated the Vitameter against U.S.P. reference oil are shown in Table 3.

Here again (Table 3) the variations from the average for each oil are quite wide, although somewhat less than those with the other procedures, the greatest variation being about 21 per cent. It can be seen that although better results are obtained when the determination is a comparison with a standard, such as the U.S.P. reference cod liver oil, the results to be expected from the use of this instrument by different operators and in different laboratories are far from being sufficiently concordant to warrant its immediate recommendation as an official method.

It should also be remembered that this work was done with materials of fairly well-known identity, where the greater part of the absorption

TABLE 2.—*Results obtained by using collaborator's procedure on untreated oil and on unsaponifiable fraction of oil*

| COLLABORATOR                        | SAMPLE 1 | SAMPLE 2                                 | SAMPLE 3                         | SAMPLE 4                         | SAMPLE 5                                       |
|-------------------------------------|----------|--|----------------------------------|----------------------------------|--|
| (a) <i>Untreated oil*</i>           |          |  |                                  |                                  |  |
| 1                                   | 815      | 3015                                     | 1290                             | 1830                             | 1225   |
| 2                                   | 1126     | 3865                                     | 1495                             | 2230                             | 1597   |
| 3                                   | 729      | 3035                                     | 1164                             | 1726                             | 1172   |
| 8                                   | 785      | 2875                                     | 1367                             | 2092                             | 1214   |
| 9                                   | 975      | 2840                                     | 1262                             | 1657                             | 1280   |
| 11                                  | 1120     | 4437                                     | 1817                             | 2451                             | 2007   |
| Average                             | 925      | 3344                                     | 1399                             | 1997                             | 1415   |
| (b) <i>Unsaponifiable fraction†</i> |          |  |                                  |                                  |  |
| 3                                   | 852      | 2812                                     | 1193                             | 1760                             | 1222   |
| 4                                   | 937      | 2804                                     | 1434                             | 1894                             | 1544   |
| 5                                   | 853      | 3276                                     | 1491                             | 1825                             | 1285   |
| 6                                   | 540      | 2597                                     | 1068                             | 1351                             | 800  |
| 11                                  | 1014     | 4015                                     | 1564                             | —                                | 1733   |
| Average                             | 839      | 3100                                     | 1350                             | 1707                             | 1316   |
| Biological Value                    | 750      | 3000<br>(U.S.P. Reference Cod Liver Oil) | 1241<br>(78% No. 1<br>22% No. 2) | 1762<br>(55% No. 1<br>45% No. 2) | 1000<br>(Composite of 21 com'l cod liver oils) |

\* The variations from the average range up to 42% in the case of these determinations made on the untreated oil.

† The five results obtained on the unsaponifiable fraction of the oils also show wide variations, the greatest deviation from the average being 39%.

TABLE 3.—*Collaborative results showing comparison with U.S.P. Reference Oil*

| COLLABORATOR     | SAMPLE 1 | SAMPLE 2                                 | SAMPLE 3                         | SAMPLE 4                         | SAMPLE 5                                       |
|------------------|----------|--|----------------------------------|----------------------------------|--|
| 1                | 890      | 2930                                     | 1280                             | 1740                             | 1320   |
| 2                | 764      | 3255                                     | 1360                             | 1980                             | 1150   |
| 3                | 872      | 2580                                     | 1247                             | 1810                             | 1196   |
| 4                | 865      | 2687                                     | 1412                             | 1816                             | 1477   |
| 5                | 825      | 3210                                     | 1235                             | 1813                             | 1250   |
| 8                | 767      | 2677                                     | 1262                             | 1899                             | 1126   |
| 9                | 1036     | 2940                                     | 1290                             | 1698                             | 1250   |
| 10               | 845      | 2819                                     | 1294                             | 1842                             | 1253   |
| Average          | 858      | 2877                                     | 1297                             | 1824                             | 1252   |
| Biological Value | 750      | 3000<br>(U.S.P. Reference Cod Liver Oil) | 1241<br>(78% No. 1<br>22% No. 2) | 1762<br>(55% No. 1<br>45% No. 2) | 1000<br>(Composite of 21 com'l cod liver oils) |

was due to vitamin A, but that in the case of unknown materials it is quite possible for substances other than vitamin A to be present and absorb light of wave length 3280 Ångstrom units. Before the adoption of an official method can be considered, it will be necessary to standardize the manipulations used in preparing the sample and the calculations by which the potency in units is derived in order that different operators can obtain concordant results, and also to determine what interfering substances may be present in the oils under examination and to devise means to eliminate the influence of such substances.

It is recommended<sup>1</sup> that further investigation of the use of the Vitaminometer for the determination of vitamin A be made.

## REPORT ON VITAMIN D

### DEVELOPMENT OF A METHOD FOR THE ASSAY OF VITAMIN D IN MILK

By WALTER C. RUSSELL (New Jersey Agricultural Experiment  
Station, New Brunswick, N. J.), *Associate Referee*

A questionnaire sent to laboratories engaged in the assay for vitamin D in milk revealed that the procedure described in Interim Announcement No. 2, 1934 Revision of the Pharmacopoeia of the United States, Tenth Decennial Revision, was being followed as the basic method, but that numerous departures from the exact procedure had been introduced. This method will be referred to hereinafter as the U.S.P. X 1934 method.

The general problem in the development of a method for the assay for vitamin D in milk is the determination of the modifications that should be introduced in the U.S.P. X 1934 method so that it will be applicable to milk and milk products. Examination of this method reveals the following principal steps: I—Depletion period; II—Assembling rats into groups for the assay period; III—Assay period; IV—Line test; V—Recording of data; and VI—Report of vitamin D potency.

In the development of a method for the assay for vitamin D in milk it appeared advisable for the present to adopt outright, or with only slight modification, certain of the above steps. Accordingly the following steps, with the slight modifications indicated, were used as described in the Interim Announcement cited above.

I—Same. II—Same except as regards the requirement concerning the number of rats of one sex in a group. Although the method requires that the number of rats of one sex in each group shall be the same, in practice this distribution is difficult to attain and apparently is not being strictly observed. Hence it is recommended that this requirement be fulfilled as

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 52 (1936).

nearly as is practicable. IV—Same. V—Same. VI—Conclusions as to the potency of a sample are to be based on the general principles expressed in the U.S.P. X 1934 procedure. The actual statement of conclusions in a test involving one assay level should be that the sample contains not less than (or less than) the number of U.S.P. X 1934 units claimed per quart.

The adaptation of the U.S.P. X 1934 method to the assay of vitamin D milk will involve changes principally under Section III, assay period, listed above.

## PROJECTS

### *1.—Comparison of 3-day with 8-day feedings of liquid milk*

The technic for cod liver oil requires the feeding of the calculated quantity of oil as 8 daily doses, separate from the ration, for the first 8 days of the assay period. This procedure can also be followed with milk, but there is the possibility of receiving the sample, especially in hot weather, in such a condition that it cannot be preserved for 8 days. This would be the minimum period of storage if the 8-day feeding procedure is followed and if all assay animals are ready at one time. Usually it is not possible to start all the rats on one day, and therefore a period of storage longer than 8 days is necessary.

If a shorter period of feeding the sample is satisfactory, one day or three days for example, a shorter period of storage could be used, there would be less handling of the sample, and the amount of time spent on an assay could be reduced. A 1-day feeding is not practical, because in some colonies it is necessary to feed as much as 25 cc. of irradiated milk. This volume can be fed as three doses, and therefore three feedings is the minimum number possible. Hence one of the projects was a comparison of 3 daily feedings of milk with 8 daily feedings, and a like comparison in case of the reference oil. The results of these trials are presented in Table 1.

The procedure of 8 daily feedings was compared with 3 daily feedings in the case of 10 samples, the work having been done by six collaborators. Three samples showed a markedly lower response when fed for 8 days as compared with 3 days. Agreement between the responses was good, but it was slightly lower for the 8-day feedings in 6 of the samples. In one instance the response after 8 days of feeding was slightly better than after 3 days. Only two comparisons of an 8-day with a 3-day feeding were made with the U.S.P. reference oil, each by a different collaborator. Lower responses were observed in both cases for the 8-day procedure. Further comparisons of these two methods of feeding should be made for milk and for the reference oil, the trials with both substances being made at the same time.



TABLE 1.—Comparison of 1-day, 3-day and 8-day feedings

| SAMPLE         | COLLABORATOR    | ASSAY<br>LEVEL | PERIOD OF<br>FEEDING | AVERAGE<br>RESPONSE * | REMARKS                     |
|----------------|-----------------|----------------|----------------------|-----------------------|-----------------------------|
| <i>Thomas</i>  |                 |                |                      |                       |                             |
|                |                 |                | <i>days</i>          |                       |                             |
| S.P. Milk      | Iowa State      | 6.6 cc.        | 3                    | 1.00                  | Fed admixed with ration     |
| "              | "               | "              | 8                    | 0.40                  | " " " "                     |
| S.L. Milk      | "               | "              | 3                    | 0.50                  | " " " "                     |
| "              | "               | "              | 8                    | 0.12                  | " " " "                     |
| S.C. Milk      | "               | "              | 3                    | 0.90                  | " " " "                     |
| "              | "               | "              | 8                    | 0.44                  | " " " "                     |
| <i>Baird</i>   |                 |                |                      |                       |                             |
| S. Milk        | Natl. Oil Prod. | 7 cc.          | 1                    | 1.40                  | CLO conc. milk              |
| "              | "               | "              | 3                    | 1.80                  | " " "                       |
| U.S.P. Oil     | "               | 30 mg.         | 1                    | 1.80                  |                             |
| "              | "               | "              | 3                    | 1.40                  |                             |
| S. Milk        | "               | 7 cc.          | 1                    | 1.70                  | CLO conc. milk              |
| "              | "               | "              | 3                    | 1.40                  | " " "                       |
| "              | "               | "              | 3                    | 1.30                  | " " "                       |
| U.S.P. Oil     | "               | 30 mg.         | 1                    | 1.40                  |                             |
| "              | "               | "              | 3                    | 1.14                  |                             |
| "              | "               | "              | 8                    | 0.84                  |                             |
| "              | "               | "              | 1                    | 1.25                  |                             |
| "              | "               | "              | 3                    | 1.25                  |                             |
| <i>Todd</i>    |                 |                |                      |                       |                             |
| Milk           | Oregon          |                | 3                    | 1.60                  |                             |
| "              | "               |                | 8                    | 1.10                  |                             |
| <i>Scott</i>   |                 |                |                      |                       |                             |
| Milk           | W.A.R.F.        |                | 3                    | 3.00                  | Irrad. milk                 |
| "              | "               |                | 8                    | 2.70                  | " "                         |
| <i>Krauss</i>  |                 |                |                      |                       |                             |
| Milk 39        | Ohio            | 8 cc.          | 3                    | 1.50                  |                             |
| "              | "               | "              | 8                    | 1.48                  |                             |
| Milk 40        | "               | "              | 3                    | 1.60                  |                             |
| "              | "               | "              | 8                    | 1.48                  |                             |
| Milk 41        | "               | "              | 3                    | 1.10                  |                             |
| "              | "               | "              | 8                    | 1.00                  |                             |
| U.S.P. Oil     | "               | 35 mg.         | 3                    | 1.20                  | 8 cc. skimmed milk also fed |
| <i>Russell</i> |                 |                |                      |                       |                             |
| Milk R-279     | New Jersey      | 9 cc.          | 3                    | 1.13                  | CLO conc. milk              |
| "              | "               | "              | 8                    | 1.30                  | " " "                       |
| U.S.P. Oil     | "               | 40 mg.         | 3                    | 0.40                  |                             |
| "              | "               | "              | 8                    | 0.25                  |                             |

\* Radii were sent to the laboratory of the associate referee, where they were scored, and an average response calculated, the following numerical values being used.

—, 0; —(±), 0.25; ±, 0.50; ±(+), 0.75; +, 1; ++(+), 1.5; ++, 2; ++++, 3; +++++, 4.

2.—*Comparison of the feeding of the milk sample incorporated with the ration with the feeding of liquid milk*

Another method of administering the sample is that of incorporating the quantity of milk to be fed with 40–50 grams of basal ration, the weight of food usually consumed in the first 7 or 8 days of the assay period. A comparison of the feeding of the milk sample incorporated in the ration with an equal volume of milk fed as liquid milk, separate from the ration, became one of the projects. A part of this project was a like comparison with the reference oil. The results of these studies are shown in Table 2.

TABLE 2.—*Comparison of the feeding of the sample admixed with the ration with feeding it separate from the ration*

| SAMPLE         | COLLABORATOR | ASSAY LEVEL | MODE OF FEEDING | PERIOD OF FEEDING | AVERAGE RESPONSE |
|----------------|--------------|-------------|-----------------|-------------------|------------------|
|                |              |             |                 | days              |                  |
| <i>Hoppert</i> |              |             |                 |                   |                  |
| Milk A         | Michigan     | 18.9 cc.    | Separate, daily | 8                 | 1.12             |
| "              | "            | "           | Incorporated    | 6–9               | 1.25             |
| Milk B         | "            | "           | Separate, daily | 8                 | 1.80             |
| "              | "            | "           | Incorporated    | 6–8               | 1.50             |
| U.S.P. Ref.    | "            | 28.4 mg.    | Separate, daily | 8                 | 0.54             |
| "              | "            | "           | Incorporated    | 8–9               | 0.75             |
| "              | "            | "           | Separate, daily | 8                 | 0.64             |
| "              | "            | "           | Incorporated    | 7–10              | 0.78             |
| <i>Russell</i> |              |             |                 |                   |                  |
| Milk R-245     | New Jersey   | 7.7 cc.     | "               | 7–9               | 0.98*            |
| "              | "            | "           | Separate, daily | 8                 | 0.95             |

\* Mixed with basal ration and stored in refrigerator for 14 days.

Three samples of milk showed essentially the same response when fed separate from the ration, daily for 8 days, and when incorporated with the ration, although in one pair of tests the response was slightly lower when the milk was fed separately and slightly higher in another. Two comparisons of the separate, daily feeding of the U.S.P. reference oil with that of incorporation in the ration were made. In both instances a slightly higher response was noted when the sample was incorporated.

3.—*Study of the effect of the feeding of the reference oil with and without the addition of non-vitamin D milk*

Since the consumption of as much as 25 cc. of milk, in the case of the irradiated product, introduces more calcium and phosphorus, and because milk may contain substances that enhance the action of an anti-

rachitic factor the suggestion has been made that a volume of whole milk or of skimmed milk, to which an antirachitic factor has not been added, be fed along with the reference oil. Only brief studies have been accomplished on this project, and they are reported in Table 3.

TABLE 3.—*Effect of the feeding of the reference oil with and without the addition of non-vitamin D milk*

| SAMPLE      | COLLABORATOR   | ASSAY LEVEL | MODE OF FEEDING | PERIOD OF FEEDING | AVERAGE RESPONSE |
|-------------|----------------|-------------|-----------------|-------------------|------------------|
|             |                | mg.         |                 | days              |                  |
| U.S.P. Ref. | <i>Hoppert</i> |             |                 |                   |                  |
| "           | Michigan       | 28.4        | Separate, daily | 8                 | 0.64             |
| "           | "              | 28.4        | "               | 8                 | 0.90*            |
| "           | "              | 28.4        | Incorporated    | 7-10              | 0.78             |
| "           | "              | 28.4        | "               | 8-9               | 0.65*            |
|             | <i>Russell</i> |             |                 |                   |                  |
| "           | New Jersey     | 40          | Separate, daily | 8                 | 0.25             |
| "           | "              | 40          | "               | 8                 | 0.68†            |

\* 18.9 cc. non-vitamin D skimmed milk fed.

† 27.6 cc. non-vitamin D skimmed milk fed.

In two comparisons in which the reference oil was fed daily for 8 days, definitely greater responses were obtained when skimmed milk was also fed. When the oil was incorporated in the ration a slightly lower response was observed in the trial in which skimmed milk was included.

#### 4.—*Study of methods of preserving milk and the effect of storage on vitamin D potency*

On account of the perishable nature of milk attention must be given to its preservation and the possible deterioration in vitamin D content during a period of storage.

Two preservatives were studied, sodium benzoate and formalin. After 22 days' storage in a refrigerator it was found that 0.4 per cent of sodium benzoate and 2 drops of formalin per quart were the smallest amounts of these substances which would maintain the milk in a satisfactory condition for feeding. The same sample of milk was assayed before the addition of the preservatives and after 22 days of storage with the preservatives. The results of these assays, along with that of the reference oil fed, are shown in Table 4.

The preliminary trials show (Table 3) that the use of sodium benzoate or formalin as preservatives and storage in a refrigerator for 22 days cause only a slight decrease, if any, in antirachitic potency.

In the New Jersey laboratory it has been possible to keep milk in a con-

TABLE 4.—*Effect of antirachitic potency of the addition of preservatives and of storage of milk in refrigerator*

| SAMPLE      | COLLABORATOR             | ASSAY<br>LEVEL | PERIOD OF<br>FEEDING | TREATMENT                | REMARKS                              |
|-------------|--------------------------|----------------|----------------------|--------------------------|--------------------------------------|
|             |                          |                | days                 |                          |                                      |
| Milk        | Guerrant<br>Pennsylvania | 8.2 cc.        | 8                    | None                     | 1.75 Before addition of preservative |
| "           | "                        | "              | 8                    | 2 drops formalin per qt. | 1.60 After 22 days of storage        |
| "           | "                        | "              | 8                    | 0.4% sod. benzoate added | 1.40 After 22 days of storage        |
| U.S.P. Ref. | "                        | 35 mg.         | 8                    | None                     | 2.50 At beginning of tests           |
| "           | "                        | "              | 8                    | "                        | 2.10 At end of tests                 |

dition satisfactory for feeding for more than a month by the addition of 2 drops of formalin and storage in a refrigerator at 0°–10° C. A determination of the effect on antirachitic potency was not made.

#### 5.—*Selection of a satisfactory reference substance for vitamin D milk*

Preliminary studies have suggested that the U.S.P. reference oil may not be a satisfactory reference substance for use with milk.

In view of the possibility that different antirachitic factors may be present in irradiated milk, milk to which a cod liver oil concentrate has been added, and in metabolized vitamin D milk (that from cows fed irradiated yeast) it will be necessary to determine whether the same assay procedure can be used for the three forms and whether the same reference substance is suitable for all forms of antirachitic milk.

### GENERAL

The selection of collaborators was made upon the basis of the replies to the questionnaire. Those who were using a procedure most nearly like the U.S.P. X 1934 method were asked to cooperate in making the several studies.

It seemed advisable to start work on several projects to determine whether they were worth further study before concentration upon any one phase of the work was begun.

A tentative procedure will first be worked out for whole, liquid milk, after which it will be applied with any necessary modifications to evaporated milk, condensed milk, dried milk, and ice cream.

A few laboratories use negative controls. This does not seem to be necessary because bones that show a cure up to and including two plus (a wide continuous line) also show the degree of rickets that prevailed at the beginning of the assay.

Spectrographic and chemical methods have been considered, but work on them is not contemplated at present because of the extremely small quantity of the vitamin that is probably present.

To date 522 rats have been used by the cooperating laboratories. The use of animals is expensive, and the results are obtained slowly, two factors that make for slow progress in the development of a method.

The variations in response emphasize the necessity of having results from a number of tests, preferably not less than ten from a laboratory, before definite conclusions can be reached.

The results so far obtained are of a preliminary nature and further investigations are necessary before a tentative method can be established. Projects that give the greatest promise should be selected and research efforts concentrated upon them. Until sufficient data have been obtained to permit the formulation of a tentative method, it is recommended<sup>1</sup> for the sake of uniformity that the U.S.P. X 1934 method be used.

The excellent spirit of cooperation shown by all those who were asked to collaborate is deeply appreciated by the associate referee.

## REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

During the past year a method for the determination of specific gravity in tomato products other than tomato paste was submitted to collaborators. This method was taken from National Canners Association Research Laboratory Bull. 27-L, pp. 24-27. It has been published in *This Journal*, 19, 98 (1936).

The results of collaborators, with one exception members of the U. S. Food and Drug Administration, are given in the table.

Studies of the tentative method for the determination of total solids in tomato products, *Methods of Analysis*, A.O.A.C., 1930, XXXV, 13, showed that low results may at times be obtained, probably due to excessively long drying on the steam bath with a temperature considerably over 70°. It also seems better to specify the size of dish in terms of maximum dry material per unit of drying surface.

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 52 (1936).

| <i>Analyst</i>                              | <i>Specific Gravity at 20°/20°</i> |                 |
|---|------------------------------------|-----------------|
|   | <i>Sample D</i>                    | <i>Sample H</i> |
| E. M. Hoshall, Baltimore                    | 1.0352                             | 1.0584          |
| D. W. McLaren, Buffalo                      | 1.0348                             | 1.0579          |
|   | (1.0351)                           | (1.0579)        |
| R. S. Pruitt, Cincinnati                    | 1.0346                             | 1.0572          |
| E. L. Ellis, Los Angeles                    | 1.0355                             | 1.0581          |
| A. H. Wells, Los Angeles                    | 1.0355                             | 1.0583          |
| J. A. Kime, San Francisco                   | 1.0352                             | 1.0591          |
| W. J. McCarthy, St. Louis                   | 1.0357                             | 1.0573          |
| J. I. Palmore, Washington                   | 1.0344                             | 1.0591          |
|   | (1.0349)                           | (1.0589)        |
| H. R. Smith, Nat. Cannery Assn., Washington | 1.0351                             | 1.0583          |

Studies of the tentative method for the determination of insoluble solids in tomato products, section 14, showed that higher results are obtained when a triple paper is used for filtering than is the case with a single paper, probably due to retention of soluble solids between papers.

Studies of methods for quality factors and fill of container for canned foods have not been completed.

The recognition of the suitability of the methods for microanalysis of tomato pulp, etc. (sections 26-29, inclusive) and their long established use, both in official circles and in the industry, make it desirable to have the method adopted as official.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the tentative method for total solids in tomato products, Chap. XXXV, sec. 13, be changed to read as follows:

Weigh a portion of the sample into a flat-bottomed dish of such size that the expected dry residue will not exceed 12 mg. per sq. cm. of drying surface. Distribute evenly in a thin layer over the bottom of the dish. Place in a vacuum oven at 70°, with release cock left partly open so that the degree of vacuum does not exceed 450 mm. of Hg and the moisture evolved is carried off rapidly. After reaching apparent dryness (approximately 1 hour) nearly close release cock and dry at 70° for 4 hours at a pressure not to exceed 100 mm.

(2) That the tentative method for the determination of insoluble solids in tomato products, sec. 14, be amended by eliminating the word "triple" in the second line.

(3) That the method suggested by the referee for the determination of specific gravity in tomato products be adopted as tentative.

(4) That studies of methods for determining quality factors and fill of container be continued.

(5) That the methods for the microanalysis of tomato pulp, etc. secs. 26-29, inclusive, be made official, final action under suspension of the rules.

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 98 (1936).

## REPORT ON SOILS AND LIMING MATERIALS

By W. H. MACINTIRE (The University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

The efforts of the referee during the past year have been exerted solely in an advisory capacity. The Associate Referees for pH values in Soils of the Arid and Semi-Arid Regions, and Soils of the Humid Region, have both submitted reports in which they recommend a preferential status for the glass electrode, but refrain from designating a specific type, because of the probability of early improvement and possible perfection. The referee concurs in the position taken by the two associate referees.

The Associate Referee for Less Common Elements has recommended the tentative adoption of two divergent types of analytical procedure for the determination of iodine in soils. This recommendation comes as the result of extensive research upon a problem for which it has been difficult to obtain any appreciable amount of collaboration. In the hands of experienced manipulators the two methods have shown results of satisfactory concordance. The referee therefore concurs in the recommendation that both methods be adopted as tentative. It is also recommended that the method for the determination of selenium in soils suggested by the Referee on Plants be adopted as tentative.

The Associate Referee on Liming Materials was unable to submit a report. His efforts have been directed toward a method to evaluate the residual occurrences of calcic materials in soils, and many data have been tabulated for publication.

REPORT ON HYDROGEN-ION CONCENTRATION  
OF ALKALINE SOILS

By P. L. HIBBARD (College of Agriculture, University of California, Berkeley, Calif.), *Associate Referee*

Following some preliminary correspondence to ascertain willingness to cooperate in the A.O.A.C. work of studying the methods for determination of hydrogen-ion concentration of alkali soils, the associate referee sent samples to seven laboratories in western states where alkali soils are common. The collaborators were asked to report results not later than October 1, and all responded. On account of the somewhat divergent results from different stations, it is apparent that further work should be done along this line before an official method can be proposed.

In Table 1 of results, averages by different methods are given. It is not supposed that these averages indicate the correct result, but they are interesting in making comparisons. Though some individual results depart widely from the averages, it is satisfactory to observe the fairly good agreement of averages obtained by the hydrogen electrode and the

glass electrode. The figures submitted by Colorado are, in general, higher than those of other stations, while the figures from Washington are mostly lower than others. This is perhaps because the standard buffer solutions used in setting the value of the voltmeter readings are materially different. This leads to the suggestion that it would be well to use a standard buffer that has been tested by some central standard agency such as the United States Bureau of Standards. Then all will have the same starting point.

The quinhydrone electrode results are more divergent. Many previous results obtained by this means at different times and places have shown considerable discrepancies. It is generally known that results given by the quinhydrone electrode on alkaline soils are frequently unreliable, especially if the pH is much above 8. It is surprising to see how good results on this set of soils of high pH were obtained by some operators with this instrument. Some of the divergent results are probably due to presence in the soils of active manganese.

A few results obtained with the antimony electrode show that it is not dependable for accurate results. This seems to be the general experience with this electrode.

The figures obtained with indicators show that they are capable of giving good results.

## APPARATUS AND PROCEDURES OF THE DIFFERENT OPERATORS

Instructions sent with the soil samples were as follows:

No definite procedure or apparatus is suggested. In general, one part of soil is to be mixed with two parts of CO<sub>2</sub>-free water and shaken for not less than 15 minutes before the test is made. (For peat, it seems preferable to use 5-10 parts of water to one of soil.) Usually it is better to let settle nearly one minute after final shaking before pouring off the top portion to be tested. In this way, one avoids getting much sand into the test vessel.

Please report single results, not averages of two or more, but each separately, to show variation in separate trials.

The different operators reported as follows:

*Arizona*.—Used hydrogen electrode and 1 part of soil to 2 parts of water. No statement was made as to procedure. Figures 0.3-0.5 pH unit higher were obtained with a soil-water ratio of 1/10.

*California, Plant Nutrition*.—Used 1 part of soil to 2 parts of water except for Soil 1 when the ratio was 1/5. The hydrogen electrode vessel used was fitted with a bottom of sintered glass through which hydrogen entered in a rapid stream, which kept the soil suspension stirred and produced equilibrium usually in less than a minute. The glass electrode was checked against 1 per cent acid potassium phthalate, pH 3.97 at 25° C.

*California, Soil Technology*.—Used two H<sup>+</sup> electrodes in the same soil suspension, so that readings from both were obtained almost simultaneously. One part of soil to 2 parts of water was used except on Soil 1, when it was 1/5. The apparatus was checked against 0.05 N acid potassium phthalate. Equilibrium was reached within 4 minutes with a bubbling electrode system.



TABLE 1.—*pH results reported by the collaborators*

|                       | SOIL | MIN.  | MAX.  | AV.   | NO.<br>DET. | QUINHYDRONE ELECTRODE |      |      | NO.<br>DET. |
|-----------------------|------|-------|-------|-------|-------------|-----------------------|------|------|-------------|
|                       |      |       |       |       |             | MIN.                  | MAX. | AV.  |             |
| 1. Arizona            | 1    |       |       |       |             |                       |      |      |             |
| H+ Electrode          | 2    | 7.41  | 7.50  | 7.45  | 2           |                       |      |      |             |
|                       | 3    |       |       | 7.60  | 1           |                       |      |      |             |
|                       | 4    |       |       | 8.03  | 1           |                       |      |      |             |
|                       | 5    | 8.15  | 8.17  | 8.16  | 2           |                       |      |      |             |
|                       | 6    |       |       | 8.84  | 1           |                       |      |      |             |
|                       | 7    | 10.13 | 10.15 | 10.14 | 2           |                       |      |      |             |
| 2. Calif., Plant Nut. | 1    | 6.69  | 6.86  | 6.77  | 2           |                       |      | 6.37 |             |
| H+ Electrode          | 2    | 7.58  | 7.85  | 7.72  | 2           |                       |      | 7.82 |             |
|                       | 3    | 7.42  | 7.54  | 7.49  | 2           |                       |      | 7.37 |             |
|                       | 4    | 8.15  | 8.25  | 8.20  | 2           |                       |      | 8.18 |             |
|                       | 5    | 8.10  | 8.23  | 8.17  | 2           |                       |      | 8.35 |             |
|                       | 6    | 8.92  | 9.08  | 9.00  | 2           |                       |      | 8.87 |             |
|                       | 7    | 10.05 | 10.07 | 10.06 | 2           |                       |      | 9+   |             |
| 3. Calif., Soil Tech. | 1    |       |       | 6.72  | 1           | 1 min. 15 min.        |      |      |             |
| H+ Electrode          | 2    |       |       | 7.50  | 1           | 6.53                  | 6.65 |      |             |
|                       | 3    |       |       | 7.65  | 1           | 5.99                  | 6.85 |      |             |
|                       | 4    |       |       | 7.99  | 1           | 7.85                  | 7.85 |      |             |
|                       | 5    |       |       | 8.19  | 1           | 7.42                  | 7.56 |      |             |
|                       | 6    |       |       | 8.98  | 1           | 7.53                  | 7.54 |      |             |
|                       | 7    |       |       | 10.12 | 1           | 8.48                  | 8.40 |      |             |
|                       |      |       |       |       |             | 9.58                  | 9.83 |      |             |
| 4. Colorado*          | 1    | 7.02  | 7.06  | 7.04  | 3           | 6.64                  | 6.74 | 6.70 | 3           |
| Glass Electrode       | 2    | 8.19  | 8.23  | 8.21  | 3           | 7.54                  | 7.62 | 7.58 | 3           |
|                       | 3    | 8.17  | 8.24  | 8.22  | 3           | 7.60                  | 7.65 | 7.63 | 3           |
|                       | 4    | 8.61  | 8.85  | 8.74  | 3           | 8.11                  | 8.15 | 8.13 | 3           |
|                       | 5    | 8.46  | 8.63  | 8.52  | 3           | 8.24                  | 8.31 | 8.27 | 3           |
|                       | 6    | 9.25  | 9.38  | 9.33  | 3           | 8.70                  | 8.75 | 8.73 | 3           |
|                       | 7    | 10.19 | 10.40 | 10.30 | 3           | 9.78                  | 9.87 | 9.82 | 3           |
| 5. Montana            | 1    | 6.64  | 6.83  | 6.72  | 3           |                       |      |      |             |
| H+ Electrode          | 2    | 7.86  | 8.07  | 7.97  | 3           |                       |      |      |             |
|                       | 3    | 7.76  | 7.83  | 7.79  | 3           |                       |      |      |             |
|                       | 4    | 8.31  | 8.34  | 8.32  | 3           |                       |      |      |             |
|                       | 5    | 8.30  | 8.45  | 8.39  | 3           |                       |      |      |             |
|                       | 6    | 9.11  | 9.12  | 9.12  | 3           |                       |      |      |             |
|                       | 7    | 10.02 | 10.06 | 10.04 | 3           |                       |      |      |             |
| 6. Nevada             | 1    | 6.82  | 7.00  | 6.90  | 6           |                       |      |      |             |
| Glass Electrode       | 2    | 7.55  | 7.56  | 7.56  | 4           |                       |      |      |             |
|                       | 3    | 7.65  | 7.75  | 7.69  | 4           |                       |      |      |             |
|                       | 4    | 8.18  | 8.20  | 8.19  | 3           |                       |      |      |             |
|                       | 5    | 8.05  | 8.22  | 8.15  | 3           |                       |      |      |             |
|                       | 6    | 8.88  | 8.91  | 8.89  | 3           |                       |      |      |             |
|                       | 7    | 10.00 | 10.01 | 10.01 | 3           |                       |      |      |             |

TABLE 1.—*pH* results reported by the collaborators (Continued)

|                 | SOIL | MIN.  | MAX.  | AV.   | NO.<br>DET. | QUINHYDRONE ELECTRODE |      |     |             |
|-----------------|------|-------|-------|-------|-------------|-----------------------|------|-----|-------------|
|                 |      |       |       |       |             | MIN.                  | MAX. | AV. | NO.<br>DET. |
| 7. Oregon       | 1    | 7.09  | 7.12  | 7.10  | 4           |                       |      |     |             |
| H + Electrode   | 2    | 7.69  | 7.75  | 7.72  | 4           |                       |      |     |             |
|                 | 3    | 7.92  | 7.93  | 7.93  | 3           |                       |      |     |             |
|                 | 4    | 8.43  | 8.49  | 8.46  | 3           |                       |      |     |             |
|                 | 5    | 8.52  | 8.59  | 8.55  | 3           |                       |      |     |             |
|                 | 6    | 9.12  | 9.25  | 9.20  | 4           |                       |      |     |             |
|                 | 7    | 10.05 | 10.09 | 10.08 | 4           |                       |      |     |             |
| 8. Washington   | 1    | 6.55  | 6.55  | 6.55  | 3           |                       |      |     |             |
| Glass Electrode | 2    | 7.00  | 7.00  | 7.00  | 3           |                       |      |     |             |
|                 | 3    | 7.15  | 7.15  | 7.15  | 3           |                       |      |     |             |
|                 | 4    | 8.10  | 8.15  | 8.13  | 3           |                       |      |     |             |
|                 | 5    | 8.05  | 8.10  | 8.08  | 3           |                       |      |     |             |
|                 | 6    | 8.85  | 8.85  | 8.85  | 3           |                       |      |     |             |
|                 | 7    | 10.10 | 10.10 | 10.10 | 3           |                       |      |     |             |

\* Colorado finds drifting of quinhydrone electrode in Soils 2, 4, 5, and 6.

TABLE 2.—Averages of the several collaborative results

|                           | SOIL NUMBERS                |      |      |      |      |      |       |
|---------------------------|-----------------------------|------|------|------|------|------|-------|
|                           | 1                           | 2    | 3    | 4    | 5    | 6    | 7     |
|                           | (By H + or Glass Electrode) |      |      |      |      |      |       |
| 1. Arizona                |                             | 7.45 | 7.60 | 8.03 | 8.16 | 8.84 | 10.14 |
| 2. California, Plant Nut. | 6.77                        | 7.72 | 7.49 | 8.20 | 8.17 | 9.00 | 10.06 |
| 4. Colorado               | 7.04                        | 8.21 | 8.22 | 8.74 | 8.52 | 9.33 | 10.30 |
| 5. Nevada                 | 6.90                        | 7.56 | 7.69 | 8.19 | 8.15 | 8.89 | 10.01 |
| 7. Washington             | 6.55                        | 7.00 | 7.15 | 8.13 | 8.08 | 8.85 | 10.10 |
| 6. Oregon                 | 7.10                        | 7.22 | 7.93 | 8.46 | 8.55 | 9.20 | 10.08 |
| 3. California, Soil Tech. | 6.72                        | 7.50 | 7.65 | 7.99 | 8.19 | 8.98 | 10.12 |
| 5. Montana                | 6.72                        | 7.97 | 7.79 | 8.32 | 8.39 | 9.12 | 10.04 |
| Average                   | 6.83                        | 7.64 | 7.69 | 8.26 | 8.28 | 9.04 | 10.11 |
|                           | (By Glass Electrode)        |      |      |      |      |      |       |
| Colorado                  | 7.04                        | 8.21 | 8.22 | 8.74 | 8.52 | 9.33 | 10.30 |
| California, Plant Nut.    | 6.74                        | 7.61 | 7.66 | 8.08 | 8.22 | 9.04 | 9.94  |
| Nevada                    | 6.90                        | 7.56 | 7.69 | 8.19 | 8.15 | 8.89 | 10.01 |
| Washington                | 6.55                        | 7.00 | 7.15 | 8.13 | 8.08 | 8.85 | 10.10 |
| Average                   | 6.81                        | 7.60 | 7.68 | 8.28 | 8.24 | 9.03 | 10.06 |
|                           | (By Quinhydrone Electrode)  |      |      |      |      |      |       |
| California, Plant Nut.    | 6.37                        | 7.82 | 7.37 | 8.18 | 8.35 | 8.87 | 9.50  |
| California, Soil Tech.    | 6.53                        | 5.99 | 7.85 | 7.42 | 7.53 | 8.48 | 9.58  |
| Colorado                  | 6.70                        | 7.58 | 7.63 | 8.13 | 8.27 | 8.73 | 9.82  |
| Average                   | 6.53                        | 7.13 | 7.62 | 7.91 | 8.06 | 8.69 | 9.63  |
| California, Plant Nut.    |                             |      |      |      |      |      |       |
| Antimony Electrode        | 6.50                        | 7.24 | 7.76 | 7.71 | 8.09 | 9.04 | 9.52  |
| Color Indicators          | 6.8                         | 7.6  | 7.8  | 8.4  | 8.4  | 9.0  | 9.5   |

*Colorado*.—Used a 1/2 ratio of soil and water with a glass electrode, the suspension being stirred just before the potential was read. No drifting was observed with this, but on four of the soils drifting occurred with the quinhydrone electrode. No statement was made as to checking the apparatus against any standard.

*Nevada*.—Used a 1/2 soil-water ratio, except on Soil 1, a 1/10 ratio, and a L. & N. glass electrode outfit. No statement was made in regard to checking the apparatus.

*Oregon*.—Used a circulating hydrogen electrode with a soil-water ratio of 1/5 except 1/10 on Soil 1, and checked against 0.05 *M* acid potassium phthalate. Equilibrium was produced in 1–2 minutes.

*Washington*.—Used a glass electrode with a soil-water ratio of 1/2 with one exception, 1/5 on Soil 1. No statement was made in regard to checking the apparatus.

The figures submitted by most of the operators show that they reproduced their own results very closely and give evidence that great care was used in making the determination.

#### CHARACTER OF THE SOILS USED FOR pH DETERMINATION

Table 3 gives an indication of the physical character of the soils studied and a rough estimate of the amounts of several of the important ions characteristic of alkali soils. Though not determined quantitatively, water-soluble sodium was known to be present in considerable amounts in all of the soils.

TABLE 3.—*Composition (rough) of 1/2 water extract of the soils, calculated to dry soils (Results expressed as p.p.m.)*

| SOIL NO.                   | CaCO <sub>3</sub> | Ca  | Na   | CO <sub>2</sub> | HCO <sub>3</sub> | SO <sub>4</sub> | Cl   |
|----------------------------|-------------------|-----|------|-----------------|------------------|-----------------|------|
| 1 Saline, peat             | 0                 | 750 | +++  | 0               | 427              | 2400            | 800  |
| 2 Saline, sandy            | 0                 | 300 | +++  | 0               | 61               | 1250            | 1275 |
| 3 Saline, clay             | 0                 | 130 | ++++ | 0               | 305              | 975             | 600  |
| 4 Calcareous, saline sandy | ++                | 250 | ++++ | 0               | 366              | 0               | 4350 |
| 5 Calcareous, saline clay  | ++++              | 110 | ++   | 0               | 610              | 35              | 100  |
| 6 Alkaline, clay           | 0                 | 0   | ++   | 60              | 854              | tr.             | 0    |
| 7 Saline, alkaline sandy   | ?                 | 0   | ++++ | 3300            | 3050             | 2000            | 7600 |

Before sending out the soil samples for cooperative work, the associate referee asked a number of questions of each collaborator. One of them was: "What limits of accuracy  $\pm$  do you think desirable for practical purposes in determining the pH of alkali soils?" Most of them replied that a  $\pm$  variation of 0.2 would be good enough. Some of the results returned from the several laboratories vary much more than 0.2, but if the general averages are taken as the correct result, many of the averages of results from each laboratory are within that limit. However, it is evident that greater accuracy is desirable. The present associate referee is not in position to continue this work, but suggests that in order to obtain greater uniformity of results in further study,<sup>1</sup> it is desirable—

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 46 (1936).

1. That precise specifications of procedure and accurate descriptions of apparatus to be used be sent with the samples.

2. That some recognized standard solution, easily obtainable and certified by the U. S. Bureau of Standards, be sent with the soil samples, so that each collaborator will have the same standard for checking the accuracy of his apparatus.

3. That the glass electrode be accepted as most reliable, and the soil suspension be mechanically stirred just before the voltmeter reading is made. Theoretically the glass electrode is more desirable than the hydrogen electrode for soil suspensions, since nothing need be added and the electrode is not affected by redox substances. But unless the instrument is well designed and properly shielded from outside electrical influences it may be practically worthless. Besides, the thin-walled glass bulbs used as electrodes are very fragile and usually somewhat bulky.

On the other hand the hydrogen electrode is sometimes more convenient and usually more rugged, but it is easily poisoned by substances sometimes present in the material being tested, so that it gives erroneous results. Besides, the hydrogen bubbling through the solution may expel carbon dioxide and thus change the *pH* of the solutions.

#### LITERATURE

McGeorge, W. T. 1935. Measurement and significance of hydroxyl-ion concentrations in alkaline-calcareous soils. *Arizona Tech. Bull.* 57. Gives a list of references. Some excerpts are appended below.

Naftel, James A. 1934. The glass electrode and its application in soil acidity determinations. *Soil Research*, 4, No. 1, p. 41. Gives a number of references.

Hibbard, P. L. 1933. Report on reaction value of alkaline soils. *J. Assoc. Official Agr. Chem.*, 16, 193-202 (1933).

W. T. McGeorge.—Presents an informative and instructive study of determination of *pH* confined to calcareous alkali soils. Not all of his conclusions apply to non-calcareous soils, but among those of interest in this connection may be mentioned:

3. Fineness of subdivision of a soil makes little difference in *pH*.

4. Moist soil fresh from the field has a lower *pH*, sometimes as much as 0.5 *pH* unit, than the same soil after drying.

8. CO<sub>2</sub> must be excluded during determination of *pH* of alkaline soils.

10. The *pH* usually decreases somewhat during 24 hours after the soils-water suspension is made with dilutions of 1/5 or more, but with 1/1 dilution little change in *pH* was observed during 72 hours.

Causes of increase of *pH* by dilutions are ionization hydrolysis and buffer effect.

Since the chief interest of McGeorge is in discovering and predicting what will be the *pH* of the soil under varying conditions of moisture and land treatment, he suggests making the determination so that probable minimum and maximum values may be found as follows: Maximum, use 1 soil to 10 water. Minimum, to 50 cc. of this same suspension add 10 cc. of 2 *M* NaCl and 10 cc. of 2 *M* CaCl<sub>2</sub>, because such highly ionized salts lower the *pH* of alkali soils materially.

A heavy shower of rain on an arid saline alkaline soil may considerably change its *pH* in a few hours.

Other points of interest in the paper are that variation in soil/water ratio is not important in determining  $pH$  of non-calcareous soils. The  $pH$  of the soil solution is lower than that of a water extract of the same soil. The potential alkalinity of a soil is not necessarily the actual found at any one time.

## REPORT ON HYDROGEN-ION CONCENTRATION OF ACID SOILS

### STUDIES OF SOIL REACTION METHODS BY THE INTERNATIONAL SOCIETY OF SOIL SCIENCE

By M. F. MORGAN (Connecticut Agricultural Experiment  
Station, New Haven, Conn.), *Associate Referee*

Since the present summer witnessed the Third Congress of the International Society of Soil Science at Oxford, England, it is timely to present a brief review of the activities of this organization toward the standardization of soil  $pH$  measurements. The associate referee has been in close touch with this work, and has attended all three of the International Congresses.

Commission II, Soil Chemistry of the International Society of Soil Science, held an interim meeting in Budapest in 1929. At that time the quinhydrone method for  $pH$  determination of soils, originally proposed by Biilman,<sup>1</sup> was already under some criticism due to the inaccuracies obtained on certain soils containing oxidizing substances, such as manganese dioxide. Hence, a committee was then appointed to study the quinhydrone and other  $pH$  methods.

This committee reported<sup>2</sup> to the Second International Congress at Leningrad in 1930, and its recommendations were adopted.<sup>3</sup> These were substantially as follows, with respect to points in question.

That a preliminary approximate measurement be made; soil and water (1:2.5) to be shaken for 1 minute, the quinhydrone then added, followed by shaking for less than 2 seconds, and the potential measured at 10 second and 60 second intervals after the introduction of the electrode and KCl bridge. When the increase in apparent  $pH$  value from 10 to 60 seconds in the preliminary measurement is less than 0.2, the soil may be regarded as suitable for the quinhydrone method. The determination should be repeated with less rapid manipulation, with 10 seconds' shaking after the quinhydrone is added, before the electrode and bridge are introduced. The final potential is to be taken at the end of 60 seconds.

When the increase in apparent  $pH$  from 10 to 60 seconds is more than 0.2, the quinhydrone method is unsuitable, but if no other is available, approximate  $pH$  values may be obtained by repeating the determination, accepting the potential obtained at the end of 10 seconds.

<sup>1</sup> *J. Agr. Sci.*, 14, 232-239 (1924).

<sup>2</sup> *Soil Research*, 2, 77-139 (1930).

<sup>3</sup> *Ibid.*, 141-144.

No proposals with respect to the glass electrode were made at that time.

Commission II then held an interim meeting in Copenhagen in 1933.<sup>1</sup> At this time Bradfield presented a resumé of a paper on the glass electrode printed in full in the official journal of the International Society.<sup>2</sup> This aroused interest in this method, and the Soil Reaction Committee was instructed to carry out a cooperative study of the glass electrode in comparison with the quinhydrone method.

This committee presented its report<sup>3</sup> to the International Society at the Oxford Congress. Its studies involved 21 soils, a number of which had previously been found to give large errors by the quinhydrone method. The glass electrodes used were of the Kerridge pattern described by Heintze,<sup>4</sup> and the MacInnes-Dole pattern described by Bradfield.<sup>2</sup>

For 12 of the soils that showed no quinhydrone drift, maximum discrepancy between 60 second quinhydrone results and glass electrode measurements at three of the laboratories was only 0.13 pH. Three other laboratories obtained somewhat poorer agreement on a few of the soils.

The nine soils showing definite potential drift between the 10 second and 60 second quinhydrone readings were checked with the glass electrode to within 0.26 pH and 0.34 pH at the Gröningen and Berlin laboratories, respectively, on the basis of the 10 second quinhydrone results. The four other collaborators found much wider discrepancies.

The glass electrode method proved satisfactory on all soils tested. Two of the collaborators, comparing the Kerridge and MacInnes-Dole types of electrodes, found them in excellent agreement.

The agreement between quinhydrone and glass electrode methods was reported as satisfactory for soils without quinhydrone drift, between the readings taken 10 and 60 seconds after the quinhydrone was added. In such cases it was again recommended that 60 second readings should be accepted.

Due to the difficulties in obtaining reproducible results on the basis of the 10 second quinhydrone potential readings, it was suggested that all soils showing quinhydrone drift be measured by the glass electrode.

A number of the members expressed themselves as favoring the use of the glass electrode in routine practice if it is to be required on the exceptional soils. Thus the necessity for maintaining equipment for both methods in good working order would be eliminated. Rapid improvements in glass electrode technic toward simplicity, ruggedness, and lower cost may be expected to encourage the present trend toward this method.

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No report on liming materials was given by the associate referee.

<sup>1</sup> *Proc. Intern. Soc. Soil Sci.*, 8, 89 (1933).

<sup>2</sup> *Soil Research*, 3, 222-246 (1933).

<sup>3</sup> *Trans. Third Intern. Cong. Soil Sci.*, 1, 127-132 (1935).

<sup>4</sup> *J. Agr. Sci.*, 24, 28-40 (1934).

## REPORT ON LESS COMMON ELEMENTS IN SOIL

By J. S. McHARGUE, *Associate Referee* and D. W. YOUNG  
(Kentucky Agricultural Experiment Station,  
Lexington, Kentucky)

During the past year further attention was given to methods for the determination of iodine in soil. This report is concerned with the volatilization and the fusion methods for this determination. The work was done by D. W. Young and the Associate Referee, in the Department of Chemistry of the Kentucky Agricultural Experiment Station. Valuable collaborative work on A.O.A.C. samples by the fusion method was obtained through the kind cooperation of W. M. Shaw and W. H. MacIntire, the Referee on Soils, at the Tennessee Experiment Station.

Since G. S. Fraps and his associates at the Texas Experiment Station, *This Journal*, 18, 314 (1935), have raised some points of controversy of the procedure previously published by the writers in *This Journal*, 18, 207 (1935), it was considered that some additional work was necessary to further confirm previous findings, to the effect that the Von Fellenberg procedure as modified by the writers gives an accurate measure of the iodine content of a soil.

The procedure that was used is a slight modification of the method published by Von Fellenberg.<sup>1</sup> It has been widely used by foreign investigators.

The modified method has been published, *This Journal*, 19, 66 (1936).

TABLE 1.—Iodine content of samples of soil expressed as p.p.m.

| COLLABORATOR     | FUSION METHOD |            | VOLATILIZATION METHOD |               |
|------------------|---------------|------------|-----------------------|---------------|
|                  | SOIL NO. 1    | SOIL NO. 2 | SOIL NO. 2            | SOIL NO. 3    |
| D. W. Young      | 16.6          | 14.2       | 14.8                  | 5.65          |
|                  | 17.9          | 14.0       | 13.2                  | 6.47          |
|                  | 17.4          | 14.7       | 15.1                  | 5.70          |
| Average          | 17.4          | 14.3       | 14.4                  | 5.94          |
| J. S. McHargue   | 17.7          | 15.2       | 14.2                  |               |
|                  | 18.5          | 14.8       | 13.8                  | Fusion method |
|                  |               | 17.3       | 14.0                  | 6.00          |
| Average          | 18.1          | 15.7       | 14.0                  |               |
| Shaw & MacIntire | 16.0          |            |                       |               |

To further test the fusion and volatilization methods, three 25 gram portions of a soil were heated in the electric furnace according to the volatilization method. The results shown in Table 1 were obtained.

<sup>1</sup> *Biochem. Z.*, 152, 116 (1924).

After being cooled the ignited residues were transferred to a porcelain mortar and finely ground, and 25 grams was placed in the porcelain boat. To the pulverized ignited soil 0.0001 gram of iodine in the form of potassium iodide was added and ignited in the electric furnace; 0.00012 gram of iodine was recovered. After the second ignition the soil was cooled and reground, and 10 grams was fused with potassium hydroxide as described in the fusion method. A perfect blank for iodine was obtained from the ignited soil residue. This experiment proved a 100 per cent recovery of the potassium iodide added and also a complete blank on the ignited residue by the fusion method.

Accordingly, the writers maintain that the iodine content of a soil can be determined accurately by the fusion method and also by the volatilization method. The latter is preferable because it is rapid and easily performed. It is also applicable to the determination of iodine in limestone rocks and phosphate rock.

#### DISCUSSION

The paper by Fraps and his coworkers contains statements with which the writers do not concur, for example, "Potassium chloride was not separated satisfactorily from the solution by alcohol, since no division of the alcohol and salt solution was found to occur, while potassium sulfate was separated sharply." It was found by the writers that when alcohol is added to a saturated aqueous solution of either potassium chloride or sulfate, salts are precipitated and that only with aqueous solutions containing potassium carbonate are immiscible phases of the aqueous and alcoholic solutions formed. Accordingly it was not found possible to form two immiscible layers with a strong aqueous solution of potassium sulfate and alcohol.

Fraps et al. also state: "Furthermore, the potassium chloride retained some of the iodine as shown by tests on known solutions, while the potassium sulfate did not." According to Seidell's book "Solubilities of Organic and Inorganic Substances," page 252, 6.2 grams of potassium iodide is soluble in 100 grams of 91 per cent alcohol at 18° C. The writer determined the solubility of relatively pure potassium iodide in pure 95, 90, and 85 per cent ethyl alcohol at room temperature, which was about 21° C., and obtained the following results:

| <i>100 mm. of<br/>alcohol</i> | <i>Dissolved grams<br/>of KI</i> |
|-------------------------------|----------------------------------|
| 95%                           | 4.024                            |
| 90%                           | 4.830                            |
| 85%                           | 9.752                            |

These facts concerning the solubility of potassium iodide and potassium chloride in pure 95 per cent ethyl alcohol afford ample proof that a mixture of the two salts can be separated quantitatively by the proper use



of the pure 95 per cent ethyl alcohol as the solvent. Accordingly, the writers do not concur in the statement that potassium iodide cannot be separated quantitatively from potassium chloride salts with the proper use of 95 per cent ethyl alcohol. They also do not approve of converting the excess of potassium hydroxide to sulfate, rather than to the chloride, because the filtrate from the silica, iron, and aluminum precipitate contains calcium compounds and upon evaporation calcium sulfate, which is not readily dissolved in hot water, will be formed in the resolution of the salts after the first series of extractions with alcohol. Consequently the insoluble calcium sulfate will retain iodine and cause lower results than when the salts are in the form of the readily water-soluble chlorides.

In neither case can all the iodine be extracted from a mixture of potassium iodide and chloride or potassium iodide and potassium sulfate by repeated treatments with 95 per cent alcohol because after the first extraction with alcohol it is necessary to dissolve the residue of salts in water and evaporate to a sludge to expose the potassium iodide molecules that are in the interior of either the chloride or sulfate crystals during the first extraction. A resolution of the residue of salts should be made at least twice.

TABLE 2.—*Study of coloration with different solvents for iodine*  
(Bausch and Lomb micro-colorimeter used)

| ADDED IODINE | IODINE RECOVERED        |                          |                           |
|--------------|-------------------------|--------------------------|---------------------------|
|              | SOLVENT CS <sub>2</sub> | SOLVENT CCl <sub>4</sub> | SOLVENT CHCl <sub>3</sub> |
| gram         | gram                    | gram                     | gram                      |
| 0.00001      | 0.00001                 | Trace of color           | Trace of color            |
| 0.00002      | 0.00002                 | 0.000018                 | 0.000018                  |
| 0.00003      | 0.000029                | 0.000028                 | —                         |
| 0.00010      | 0.000098                | 0.000100                 | { 0.000099<br>0.000100    |
| 0.00020      | 0.000200                | 0.000188                 | { 0.000200<br>0.000216    |
| 0.00030      | 0.000300                | { 0.000302<br>0.000259   | { 0.000304<br>0.000300    |

Fraps et al. object to the use of carbon disulfide as a solvent for iodine because they say it is volatile and has a bad odor. Very little comparative work has been done to ascertain the best solvent for the micro determination of iodine. So far as can be ascertained from the literature, carbon disulfide, carbon tetrachloride, and chloroform have been used by different investigators. The results (Table 2) show the error obtained on unknown solutions by the use of these solvents when a Bausch and Lomb micro-colorimeter was used. With quantities of 0.00001 gram of iodine in 10 ml. of water carbon disulfide gave the most sensitive coloration, due

to the coefficient of distribution of iodine between solvent and water. In this respect carbon disulfide is by far the best solvent. Iodine at 25° C. is nine times more soluble in carbon disulfide than in carbon tetrachloride, and therefore when a small amount of iodine is present, a smaller volume of carbon disulfide will extract all the iodine and give a more intense color, which makes a more accurate determination possible. It is also a well-known fact that pure carbon disulfide is odorless. Furthermore, if the readings are made as soon as the iodine color is developed, no appreciable error is caused by volatilization of carbon disulfide at ordinary laboratory temperatures during the short time required to make a reading with a micro-colorimeter.

In reply to the statement that the use of potassium sulfite for the reduction of iodate to iodide causes a loss of iodine, the results from a number of experiments show that in no case was there a loss of iodine when an adequate amount of potassium sulfite, 0.01 gram, was used. Excessive amounts of this salt cause a cloudiness of the carbon disulfide and make impossible a sharp clear color and an accurate reading.

The only point raised in which the writers concur is to the effect that a colorimetric determination for small quantities of iodine is more accurate than a determination made by titration. Reference was made to a "Direct Method," but no description of or results by such a method are presented in the paper by Fraps et al.

It is difficult to find a high grade of potassium hydroxide and potassium nitrite free from iodine. Accordingly if only the commercial grade of these two chemical reagents was used by Fraps et al. it is quite probable that they contained iodine.

Pure reagents and adequate equipment are essential for the determination of iodine in soil. The fusion of the soil should be complete, but it is not necessary to heat the crucible to redness. The precipitate of silica, iron, and aluminum hydroxides must be thoroughly washed with hot water. The sludge of salts obtained by evaporation of the filtrate containing the potassium chloride and potassium iodide should be hot when the first portion of 95 per cent alcohol is added, and the salts should be rubbed and stirred until room temperature is attained. The salts assume a pasty consistency by this treatment, which is a desirable form for the solution of the potassium iodide in the alcohol.

The ignition temperature of the final residue of potassium iodide, which always contains traces of organic matter, must not exceed 450° C., and this temperature can be most easily controlled in an electric furnace having a pyrometer attachment, otherwise over-heating and a loss of iodine is likely to occur. Thorough extraction with alcohol and the proper temperature of ignition of the final residue are of great importance in the fusion method. The volatilization method is the more desirable procedure, and the original cost of the furnace and the upkeep are reasonable.

It is recommended<sup>1</sup> that the fusion method for the determination of iodine in soil be adopted as tentative method No. 1 and that the combustion method be adopted as tentative method No. 2 and that further collaborative work be done.

## REPORT ON FERTILIZERS

By G. S. FRAPS (Texas Agricultural Experiment Station,  
College Station, Texas), *Referee*

Methods for the analysis of fertilizers are yearly becoming more and more complex. While work is still required on the estimation of nitrogen, phosphoric acid, and potash, additional determinations are being requested for other elements now being used as fertilizers. It seems desirable to divide the work on secondary fertilizing elements, such as manganese and magnesium, from that on acid-forming fertilizers. It may be necessary to subdivide the work on secondary fertilizing elements still further.

Some differences of opinion have arisen in various states with respect to the method for ascertaining whether a fertilizer is acid-forming or nonacid-forming. To avoid further confusion and to clear up the situation, it seems desirable to adopt a method for such purposes as tentative at this meeting and to give further study to details of the method in subsequent years.

After many years of study, the Association is now ready to adopt a method for potash that recovers all the soluble potash added in mixing the fertilizer. Much credit is due to H. R. Kraybill, Associate Referee on Potash, for the excellent work he has done in devising and testing the method.

E. W. Magruder suggested some editorial changes in the methods for the activity of nitrogen. These changes have the approval of the Referee and of J. B. Smith, who was Associate Referee on Nitrogen Activity Methods until the work was completed. The editors of *Methods of Analysis* have already been authorized to make such editorial changes, so no further action is necessary.

The recommendations<sup>2</sup> of the associate referees given in their reports are endorsed, and the associate referees are commended for the excellent work they are doing.

The referee recommends that the work of the Associate Referee on High Analysis Fertilizers be divided between two associate referees, one to be the Associate Referee on Magnesium and Manganese and the other the Associate Referee on Nonacid-forming Fertilizers.

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 46 (1936).

<sup>2</sup> *Ibid.*, 49.

## REPORT ON PHOSPHORIC ACID

## THE FILTRATION OF CITRATE-INSOLUBLE RESIDUES

By WILLIAM H. ROSS, *Associate Referee*, and JOHN O. HARDESTY (Fertilizer Investigations), Bureau of Chemistry and Soils, Washington, D.C.

In the original Fresenius, Neubauer, and Luck method<sup>1</sup> for determining citrate-insoluble  $P_2O_5$  the washed sample is digested with frequent shaking in neutral ammonium citrate solution at 30°–35° C. for one-half hour, filtered, and washed. The  $P_2O_5$  in the residue is then determined for total phosphoric acid. No special directions are given for making the filtration, and the kind of filter to use in filtering off the citrate-insoluble residue is not specified. At the first meeting of this Association, in 1884, this method was adopted with the variation that the digestion in neutral ammonium citrate solution be made at 65° C.<sup>2</sup> No special filter was recommended other than that a plaited porous filter be used in the analysis of materials that are difficult to filter. It soon became evident, however, that certain phosphatic materials have a tendency to pass through porous filters in an undissolved state, giving rise to a cloudy filtrate and low results for citrate-insoluble  $P_2O_5$  that vary in proportion to the undissolved phosphate lost in this way. The difficulty of obtaining clear filtrates has been increased in recent years by the ammoniation of superphosphate and by the use of raw phosphate rock as a filler in fertilizer mixtures. Attempts have accordingly been made to improve the method of filtering citrate-insoluble residues, with the result that various types of filters and filter papers are now in use in different laboratories. A study of the effectiveness of the types of filter papers used in various control and commercial laboratories was made by Ford and Kraybill<sup>3</sup> in 1932. It was found that while some of the filters studied are suitable for use in the analysis of the usual commercial fertilizers, others are very unsatisfactory.

A modified Shimer filter as applied by Magruder in the analysis of phosphates was described by Kilgore<sup>4</sup> as Referee on Phosphoric Acid at the fifteenth meeting of this Association in 1898. This filter has the advantage that the mat and insoluble residue can be readily displaced from the filter tube by means of a wire attached to the bottom of the porcelain plate on which the mat is deposited.

The Shimer filter<sup>5</sup> as further modified by Moore<sup>6</sup> was used by MacIntire and Hardin<sup>7</sup> in a comparative study of the different solutions employed

<sup>1</sup> *Z. anal. Chem.*, 10, 133 (1871).

<sup>2</sup> *Proc. 1st Ann. Meeting of the A.O.A.C.*, 1884, p. 5.

<sup>3</sup> *This Journal*, 15, 653 (1932).

<sup>4</sup> *U. S. Dept. Agr. Bur. Chem. Bull.*, 56, 46 (1899).

<sup>5</sup> *J. Am. Chem. Soc.*, 27, 287 (1905).

<sup>6</sup> *Arthur H. Thomas Co. Cat.*, 1931 ed., p. 382.

<sup>7</sup> *This Journal*, 18, 297 (1935).

as washing reagents for the citrate-insoluble residues. It was found that clearer filtrates were obtained when the residues were washed with a 5 per cent ammonium nitrate solution, or with ammonium citrate solutions of varying concentrations, than when the residues were washed with water. It was also claimed by MacIntire, Jones, and Hardin<sup>1</sup> that the Shimer filter with a mat of paper pulp is suitable for use in the determination of water-soluble  $P_2O_5$  and that it has a distinct advantage of speed over the method of washing as directed in the official method.

The present report summarizes the results secured in a collaborative study of the relative merits of different methods of filtering and washing the citrate-insoluble residues obtained in the analysis of various types of phosphatic materials and mixtures.

The samples submitted to the collaborators were the following:

1. *Ammoniated superphosphate*, containing 6.53% of ammonia.
2. *Calcined phosphate*, prepared by heating phosphate rock in the presence of water vapor, as described by Marshall, Reynolds, Jacob, and Rader.<sup>2</sup>
3. *Triple superphosphate*, a product of the Tennessee Valley Authority.
4. *Mixed fertilizer* (3-10-5), a mixture having the same composition as the average mixture now used in this country except that the filler used consists entirely of natural colloidal phosphate.
5. *Mixed fertilizer* 0.676(6-8-4). One of several mixtures used by the Committee on Fertilizer Reaction, American Society of Agronomy, in a study of the relative neutralizing value of dolomite and phosphate rock. It contains about 40% of phosphate rock.
6. *Mixed fertilizer* (6-9-4). The dolomite in this mixture represents the maximum that is likely to be used in any fertilizer. The mixture was maintained at a temperature of 60° C. and a moisture content of 7% for one month before being distributed to the collaborators. Sufficient 100-mesh sand was added to the mixture at the end of the curing period to compensate for loss of carbon dioxide and other volatile matter. Some loss of ammonia occurred during the curing period, and the mixture became alkaline in reaction. The phosphate used in this mixture was the same as Sample 3.

The fertilizer formulas of the mixed fertilizers are as follows:

| MATERIAL                      | MIXED FERTILIZER |               |         |
|-------------------------------|------------------|---------------|---------|
|                               | (3-10-5)         | 0.676 (6-8-4) | (6-9-4) |
| Superphosphate                | 1100             | 816           | —       |
| Triple superphosphate         | —                | —             | 407     |
| Potassium chloride            | 200              | 135           | 220     |
| Ammonia, 3% of superphosphate | 33               | —             | —       |
| Ammonium sulfate              | 100              | 583           | 581     |
| Sodium nitrate                | 33               | —             | —       |
| Organic ammoniate             | 100              | —             | —       |
| Magnesium sulfate, hydrated   | —                | 122           | 118     |
| Dolomite                      | 94               | —             | 674     |
| Raw phosphate                 | 340              | 1300          | —       |
|                               | 2000             | 2956          | 2000    |

<sup>1</sup> *This Journal*, 18, 301 (1935).

<sup>2</sup> *Ind. Eng. Chem.*, 27, 205 (1935).

## DIRECTIONS FOR ANALYSIS

1. Determine citrate-insoluble  $P_2O_5$  in each of the standard samples as directed in *Methods of Analysis, A O.A.C.*, 1930, for acidulated samples, p. 17, sec. 14 (a), using Whatman filter No. 2 to filter off the citrate-insoluble residues, and wash with water at 65° C.

2. Repeat the determinations, using Whatman filter No. 5.

3. Repeat the determinations, using Schleicher and Schüll filter No. 589, blue ribbon.

4. Repeat the determinations, using Schleicher and Schüll filter No. 602.

5. Repeat the determinations, using Shimer filters with a mat of filter pulp. The filter pulp is most conveniently prepared from Schleicher and Schüll's No. 292 filter pulp disks. When these are not available, prepare the filter pulp by tearing from five to eight 9 cm. filters, or the equivalent of sheet filters, into shreds; place the pieces in a 500 cc. Erlenmeyer flask, add 250 cc. of hot water, close the flask with a rubber stopper, and, under the protection of a towel, shake vigorously until the paper is reduced to a pulp.

6. Repeat the determinations, using the filter that was found to be the most satisfactory, and wash with a 5% ammonium citrate solution at 65° C.

7. Repeat the determinations, using the filter that was found to be the most satisfactory and wash with a 5% ammonium nitrate solution at 65° C.

It is suggested—

1. That the tests with the different filters be made at the same time in order that they may be more readily compared.

2. That a record be made of the filtrates and washings that come through clear and of those that come through cloudy.

3. That an estimate be made of the time taken to make the filtration and washing in the different determinations.

4. That special care be taken to insure that the ammonium citrate solution used in the work is strictly neutral.

5. That the filtrations be made with suction with the use of either a Büchner funnel or an ordinary glass funnel with platinum cone in all determinations that do not call for a Shimer funnel.

## COLLABORATORS

The following analysts collaborated in this work:

1. Allen, H. R. and Gault, Lelah, Univ. of Kentucky, Lexington, Ky.
2. Austin, W. R., Armour Fertilizer Works, Nashville, Tenn.
3. Butt, C. A. and Hammett, A. M., Intern. Agr. Corp., East Point, Ga.
4. Byers, C. R., Armour Fertilizer Works, Carteret, N.J.
5. Caldwell, R. D., Armour Fertilizer Works, Atlanta, Ga.
6. Charlton, R. C., American Agr. Chem. Co., Baltimore, Md.
7. Dulin, T. G., Georgia Agr. Exp. Sta., Experiment, Ga.
8. Farr, Thad D., Tennessee Valley Authority, Wilson Dam, Ala.
9. Ford, O. W., Indiana Agr. Exp. Sta., Lafayette, Ind.
10. Hardesty, John O., Bur. of Chemistry and Soils, Washington, D.C.
11. Hardin, L. J., Tennessee Agr. Exp. Sta., Knoxville, Tenn.
12. Howes, C. Clifton, Davison Chemical Co., Baltimore, Md.
13. Jones, R. L., Armour Fertilizer Works, Chicago Heights, Ill.
14. Koch, R. C., Swift and Co. Fertilizer Works, Chicago, Ill.
15. Lamb, R. H., Darling and Co., St. Louis, Mo.
16. Martin, James B., Bur. of Plant Industry, Washington, D.C.
17. Montague, H. S., Mississippi State College, State College, Miss.
18. Neu, Rudolph, Armour Fertilizer Works, Jacksonville, Fla.

19. Ryder, W. A., F. S. Royster Guano Co., Norfolk, Va.  
 20. Savana, L. J., Armour Fertilizer Works, New Orleans, La.  
 21. Shuey, P. McG., Shuey and Co., Savannah, Ga.

## RESULTS OF ANALYSIS

Table 1 summarizes the results reported by the collaborators for citrate-insoluble  $P_2O_5$  in the standard samples when using different types of filters and filter papers. The results obtained when the citrate-insoluble residues were washed with the reagents indicated are given in Table 2.

TABLE 1.—*Per cent citrate-insoluble  $P_2O_5$  in standard phosphate samples as determined with different types of filters*

| COLLABORATORS                    | WHATMAN FILTER    |                   | SCHLEICHER AND SCHÜLL FILTER |                   | SHIMER<br>FILTER  | MEAN<br>FOR ALL<br>FILTERS |
|----------------------------------|-------------------|-------------------|------------------------------|-------------------|-------------------|----------------------------|
|                                  | NO. 2             | NO. 5             | NO. 589                      | NO. 602           |                   |                            |
| <i>Ammoniated Superphosphate</i> |                   |                   |                              |                   |                   |                            |
| 1                                | 4.82 <sup>a</sup> | 4.90              | 4.85                         | 4.87              | 4.67 <sup>a</sup> | 4.82                       |
| 3                                | 5.35 <sup>a</sup> | 5.20 <sup>a</sup> | 5.26 <sup>a</sup>            | 5.35 <sup>a</sup> | 5.21 <sup>a</sup> | 5.27                       |
| 6                                | 4.37              | 4.43              | 4.46                         | 4.36              | 4.46              | 4.42                       |
| 7                                | —                 | 5.72              | —                            | —                 | 5.08 <sup>a</sup> | 5.40                       |
| 8                                | 4.77              | 4.66              | 4.72                         | —                 | 4.17              | 4.58                       |
| 9                                | 5.11              | 4.70              | 4.78                         | 5.11              | 4.58              | 4.86                       |
| 10                               | 5.40 <sup>a</sup> | 5.56              | 5.74                         | 5.87              | 5.21 <sup>a</sup> | 5.56                       |
| 11                               | 4.25              | 4.50              | —                            | —                 | 4.00              | 4.25                       |
| 12                               | 4.99              | 5.03              | 4.98 <sup>a</sup>            | 5.69              | 5.53 <sup>a</sup> | 5.24                       |
| 14                               | 4.92 <sup>a</sup> | 4.97 <sup>a</sup> | 4.85 <sup>a</sup>            | 5.07 <sup>a</sup> | 5.12 <sup>a</sup> | 4.99                       |
| 15                               | 5.23              | 5.16              | 5.26                         | 5.05              | 5.00 <sup>a</sup> | 5.14                       |
| 16                               | 5.78 <sup>a</sup> | 5.91 <sup>a</sup> | 5.99 <sup>a</sup>            | 5.95 <sup>a</sup> | 5.72 <sup>a</sup> | 5.87                       |
| 17                               | 4.68              | 4.65              | 4.65                         | —                 | 4.35              | 4.58                       |
| 19                               | 5.50              | 5.70              | 5.50                         | 5.40              | 5.40              | 5.50                       |
| 21                               | 5.13              | 5.03              | 5.11                         | 5.07              | 5.11 <sup>a</sup> | 5.09                       |
| Mean                             | 5.02              | 5.05              | 5.09                         | 5.25              | 4.91              | 5.04                       |
| <i>Calcined Phosphate</i>        |                   |                   |                              |                   |                   |                            |
| 1                                | 3.35              | 3.37              | 3.40                         | 3.40              | 3.32              | 3.37                       |
| 2                                | 3.23              | 3.21              | 3.21                         | 3.12              | 3.31              | 3.22                       |
| 3                                | 3.79 <sup>a</sup> | 3.79 <sup>a</sup> | 3.72 <sup>a</sup>            | 3.77 <sup>a</sup> | 3.78 <sup>a</sup> | 3.77                       |
| 4                                | 3.60              | 3.55              | —                            | —                 | 3.55              | 3.57                       |
| 5                                | 3.60              | 3.48              | 3.48                         | 3.41              | 3.42              | 3.48                       |
| 6                                | 3.37              | 3.33              | 3.42                         | 3.31              | 3.39              | 3.36                       |
| 7                                | —                 | 3.34              | 3.59                         | —                 | 3.53 <sup>a</sup> | 3.49                       |
| 8                                | 3.43              | 3.61              | 3.37                         | —                 | 3.32              | 3.43                       |
| 9                                | 3.44              | 3.94              | 4.13                         | 3.57              | 3.53              | 3.72                       |
| 10                               | 3.61 <sup>a</sup> | 3.42 <sup>a</sup> | 3.58                         | 3.61              | 3.94              | 3.63                       |
| 11                               | 3.50              | 3.55              | 3.43                         | —                 | 3.43              | 3.48                       |
| 12                               | 3.45              | 3.61              | 3.49                         | 3.89              | 4.41 <sup>a</sup> | 3.57                       |
| 13                               | 3.65              | 3.75              | 3.65                         | 3.65              | 3.65              | 3.67                       |
| 14                               | 3.43 <sup>a</sup> | 3.51 <sup>a</sup> | 3.44 <sup>a</sup>            | 3.42 <sup>a</sup> | 3.56              | 3.47                       |

TABLE 1.—Per cent citrate-insoluble  $P_2O_5$  in standard phosphate samples as determined with different types of filters (continued)

| COLLABORATORS                   | WHATMAN FILTER    |                   | SCHLEICHER AND SCHULL FILTER |                   | SHIMER FILTER     | MEAN FOR ALL FILTERS |
|---------------------------------|-------------------|-------------------|------------------------------|-------------------|-------------------|----------------------|
|                                 | NO. 2             | NO. 5             | NO. 589                      | NO. 602           |                   |                      |
| 15                              | 3.19              | 3.16              | 3.23                         | 3.12              | 3.23 <sup>a</sup> | 3.19                 |
| 16                              | 3.85 <sup>a</sup> | 4.10 <sup>a</sup> | 3.89 <sup>a</sup>            | 3.71 <sup>a</sup> | 3.90 <sup>a</sup> | 3.89                 |
| 17                              | 3.39              | 3.40              | 3.40                         | —                 | 3.42              | 3.40                 |
| 18                              | 3.15              | 3.18              | 2.94                         | 3.20              | 3.20              | 3.13                 |
| 19                              | 3.68              | 3.70              | 3.65                         | 3.65              | 3.65              | 3.67                 |
| 20                              | 3.62              | 3.32              | 3.44                         | 3.59              | 3.32              | 3.46                 |
| 21                              | 3.77              | 3.75              | 3.53                         | 3.53              | 3.61 <sup>a</sup> | 3.64                 |
| Mean                            | 3.51              | 3.53              | 3.50                         | 3.50              | 3.55              | 3.51                 |
| <i>Triple Superphosphate</i>    |                   |                   |                              |                   |                   |                      |
| 1                               | 2.80 <sup>a</sup> | 2.80 <sup>a</sup> | 2.92                         | 2.92              | 2.22 <sup>b</sup> | 2.73                 |
| 2                               | 1.78 <sup>a</sup> | 2.09 <sup>a</sup> | 2.07 <sup>a</sup>            | 2.01 <sup>a</sup> | 2.10 <sup>a</sup> | 2.01                 |
| 3                               | 3.03 <sup>a</sup> | 3.01 <sup>a</sup> | 2.92 <sup>b</sup>            | 2.98 <sup>b</sup> | 2.85 <sup>b</sup> | 2.96                 |
| 4                               | 2.10 <sup>a</sup> | 2.15 <sup>a</sup> | 2.70 <sup>a</sup>            | 2.52 <sup>a</sup> | 2.35 <sup>a</sup> | 2.36                 |
| 5                               | 2.47              | 2.27 <sup>a</sup> | 2.03 <sup>a</sup>            | 2.80              | 2.37              | 2.39                 |
| 6                               | 2.14 <sup>b</sup> | 2.06 <sup>b</sup> | 2.51 <sup>a</sup>            | 2.60 <sup>a</sup> | 2.61 <sup>a</sup> | 2.38                 |
| 7                               | —                 | 3.13              | 3.12                         | —                 | 1.98 <sup>a</sup> | 2.74                 |
| 8                               | 2.82 <sup>a</sup> | 2.62 <sup>a</sup> | 2.80                         | —                 | 1.85 <sup>a</sup> | 2.62                 |
| 9                               | 3.13              | 3.21              | 3.06                         | 2.27              | 2.46 <sup>a</sup> | 2.83                 |
| 10                              | 2.53 <sup>b</sup> | 2.46 <sup>b</sup> | 2.85 <sup>a</sup>            | 3.13 <sup>a</sup> | 2.46 <sup>b</sup> | 2.69                 |
| 11                              | 2.85 <sup>a</sup> | 2.81 <sup>a</sup> | —                            | —                 | 2.50 <sup>a</sup> | 2.72                 |
| 12                              | 2.79 <sup>a</sup> | 3.21              | 3.18                         | 2.97              | 1.49 <sup>a</sup> | 2.73                 |
| 13                              | 2.45 <sup>a</sup> | 2.45 <sup>a</sup> | 2.55 <sup>a</sup>            | 2.75 <sup>b</sup> | 2.15 <sup>b</sup> | 2.47                 |
| 14                              | 1.81              | 2.09              | 1.96                         | 2.10              | 2.19              | 2.03                 |
| 15                              | 2.66 <sup>a</sup> | 2.74              | 2.76                         | 2.70 <sup>a</sup> | 2.69 <sup>b</sup> | 2.71                 |
| 16                              | 1.96 <sup>b</sup> | 2.30 <sup>b</sup> | 2.79 <sup>b</sup>            | 2.53 <sup>b</sup> | 2.46 <sup>b</sup> | 2.41                 |
| 17                              | 2.15 <sup>b</sup> | 2.17 <sup>b</sup> | 2.65 <sup>b</sup>            | —                 | 2.19 <sup>b</sup> | 2.29                 |
| 18                              | 2.38 <sup>b</sup> | 2.36 <sup>b</sup> | 2.26 <sup>b</sup>            | 2.43 <sup>b</sup> | 2.13 <sup>b</sup> | 2.49                 |
| 19                              | 3.10 <sup>a</sup> | 3.20 <sup>a</sup> | 3.10 <sup>a</sup>            | 3.10 <sup>a</sup> | 2.70 <sup>a</sup> | 3.04                 |
| 20                              | 2.56              | 2.54              | 2.54                         | 2.66              | 2.41              | 2.54                 |
| 21                              | 2.77              | 2.62 <sup>a</sup> | 2.78 <sup>a</sup>            | 2.72              | 2.46 <sup>b</sup> | 2.67                 |
| Mean                            | 2.51              | 2.59              | 2.68                         | 2.66              | 2.32              | 2.56                 |
| <i>Mixed Fertilizer(3-10-5)</i> |                   |                   |                              |                   |                   |                      |
| 1                               | 4.45              | 4.50              | 4.50                         | 4.47              | 4.35 <sup>a</sup> | 4.45                 |
| 2                               | 4.16 <sup>a</sup> | 4.14 <sup>a</sup> | 4.29 <sup>a</sup>            | 4.16 <sup>a</sup> | 4.35 <sup>a</sup> | 4.22                 |
| 3                               | 4.83 <sup>a</sup> | 4.79 <sup>a</sup> | 4.81 <sup>a</sup>            | 4.82 <sup>a</sup> | 4.86 <sup>a</sup> | 4.82                 |
| 4                               | 4.40              | 4.40              | 4.50                         | 4.45              | 4.35              | 4.42                 |
| 5                               | 4.30              | 4.57              | 4.26 <sup>a</sup>            | 4.35              | 4.41              | 4.38                 |
| 6                               | 4.45 <sup>a</sup> | 4.49 <sup>a</sup> | 4.57                         | 4.52              | 4.53              | 4.51                 |
| 7                               | —                 | 4.27              | 4.66                         | —                 | 4.48 <sup>a</sup> | 4.45                 |
| 8                               | 4.69              | 4.69              | 4.69                         | —                 | 4.27              | 4.59                 |
| 9                               | 4.64              | 4.58              | 4.93                         | 4.44              | 4.06 <sup>a</sup> | 4.53                 |
| 10                              | 4.91 <sup>a</sup> | 4.84 <sup>a</sup> | 4.98                         | 5.11 <sup>a</sup> | 5.05 <sup>a</sup> | 4.98                 |
| 11                              | 4.50              | 4.60              | —                            | —                 | 4.40              | 4.50                 |
| 12                              | 4.55 <sup>a</sup> | 4.71              | 4.35                         | 4.93              | 4.49 <sup>a</sup> | 4.61                 |



TABLE 1.—Per cent citrate-insoluble  $P_2O_5$  in standard phosphate samples as determined with different types of filters (continued)

| COLLABORATORS                         | WHATMAN FILTER     |                    | SCHLEICHER AND SCHÜLL FILTER |                    | SHIMMER FILTER     | MEAN FOR ALL FILTERS |
|---------------------------------------|--------------------|--------------------|------------------------------|--------------------|--------------------|----------------------|
|                                       | NO. 2              | NO. 5              | NO. 589                      | NO. 602            |                    |                      |
| 13                                    | 4.65               | 4.60               | 4.55                         | 4.50               | 4.60               | 4.58                 |
| 14                                    | 4.35 <sup>a</sup>  | 4.53 <sup>a</sup>  | 4.48 <sup>a</sup>            | 4.45 <sup>a</sup>  | 4.61 <sup>a</sup>  | 4.48                 |
| 15                                    | 4.26               | 4.30               | 4.26                         | 4.37               | 4.26               | 4.29                 |
| 16                                    | 4.34 <sup>a</sup>  | 4.51 <sup>a</sup>  | 4.67 <sup>a</sup>            | 4.54 <sup>a</sup>  | 4.62 <sup>a</sup>  | 4.54                 |
| 17                                    | 4.55               | 4.57               | 4.69                         | —                  | 4.64               | 4.61                 |
| 18                                    | 4.38 <sup>a</sup>  | 4.40 <sup>a</sup>  | 4.40 <sup>a</sup>            | 4.49 <sup>a</sup>  | 4.43 <sup>a</sup>  | 4.42                 |
| 19                                    | 4.60               | 4.60               | 4.55                         | 4.70               | 4.60               | 4.61                 |
| 20                                    | 4.30               | 4.25               | 4.35                         | 4.15               | 4.37               | —                    |
| 21                                    | 4.49               | 4.49               | 4.45                         | 4.39               | 4.54 <sup>b</sup>  | 4.47                 |
| Mean                                  | 4.49               | 4.52               | 4.55                         | 4.52               | 4.49               | 4.52                 |
| <i>Mixed Fertilizer 0.676 (6-8-4)</i> |                    |                    |                              |                    |                    |                      |
| 1                                     | 12.60 <sup>a</sup> | 12.80              | 12.75                        | 12.63              | 12.60 <sup>b</sup> | 12.68                |
| 2                                     | 12.58 <sup>a</sup> | 12.25 <sup>a</sup> | 12.63 <sup>a</sup>           | 12.46 <sup>a</sup> | 12.25 <sup>a</sup> | 12.43                |
| 3                                     | 12.89 <sup>a</sup> | 12.87 <sup>a</sup> | 12.72 <sup>a</sup>           | 12.87 <sup>a</sup> | 12.89 <sup>a</sup> | 12.85                |
| 4                                     | 13.12 <sup>a</sup> | 12.90 <sup>a</sup> | —                            | —                  | 12.90              | 12.97                |
| 5                                     | 12.76              | 13.03              | 12.53                        | 12.88              | 12.48              | 12.74                |
| 6                                     | 12.56 <sup>b</sup> | 12.40 <sup>b</sup> | 12.68 <sup>a</sup>           | 12.69 <sup>a</sup> | 12.82 <sup>a</sup> | 12.63                |
| 7                                     | —                  | 13.21              | 13.13                        | —                  | 13.15 <sup>a</sup> | 13.16                |
| 8                                     | 13.10              | 13.07              | 13.10                        | —                  | 12.78              | 13.01                |
| 9                                     | 12.73              | 13.27              | 13.20                        | 13.25              | 12.50 <sup>a</sup> | 12.99                |
| 10                                    | 13.94 <sup>a</sup> | 13.60 <sup>a</sup> | 14.33 <sup>a</sup>           | 13.89 <sup>a</sup> | 14.14 <sup>a</sup> | 13.98                |
| 11                                    | 12.80              | 12.75              | —                            | —                  | 12.63              | 12.73                |
| 12                                    | 13.13 <sup>a</sup> | 13.81 <sup>a</sup> | 13.40 <sup>a</sup>           | 13.53              | 12.69              | 13.31                |
| 13                                    | 12.90 <sup>a</sup> | 12.85 <sup>a</sup> | 12.85 <sup>a</sup>           | 12.90 <sup>a</sup> | 12.40 <sup>b</sup> | 12.78                |
| 14                                    | 13.04 <sup>a</sup> | 13.19 <sup>a</sup> | 12.95 <sup>a</sup>           | 12.87 <sup>a</sup> | 13.15              | 12.84                |
| 15                                    | 12.30 <sup>a</sup> | 12.23 <sup>a</sup> | 12.37                        | 12.33 <sup>a</sup> | 12.23 <sup>a</sup> | 12.29                |
| 16                                    | 13.40 <sup>a</sup> | 13.05 <sup>a</sup> | 13.07 <sup>a</sup>           | 13.18 <sup>a</sup> | 13.28 <sup>a</sup> | 13.20                |
| 17                                    | 13.25 <sup>a</sup> | 13.32 <sup>a</sup> | 13.35 <sup>a</sup>           | —                  | 13.24 <sup>a</sup> | 13.29                |
| 18                                    | 11.78 <sup>b</sup> | 12.13 <sup>b</sup> | 12.02 <sup>b</sup>           | 12.31 <sup>b</sup> | 12.15 <sup>b</sup> | 12.08                |
| 19                                    | 13.15 <sup>a</sup> | 13.20 <sup>a</sup> | 13.05 <sup>a</sup>           | 13.15 <sup>a</sup> | 12.90 <sup>a</sup> | 13.09                |
| 21                                    | 12.56 <sup>b</sup> | 12.73 <sup>b</sup> | 13.00 <sup>b</sup>           | 12.88 <sup>b</sup> | 12.75 <sup>b</sup> | 12.78                |
| Mean                                  | 12.87              | 12.93              | 12.95                        | 12.92              | 12.80              | 12.89                |
| <i>Mixed Fertilizer (6-9-4)</i>       |                    |                    |                              |                    |                    |                      |
| 2                                     | 0.39 <sup>a</sup>  | 0.47 <sup>a</sup>  | 0.47 <sup>a</sup>            | 0.39 <sup>a</sup>  | 0.45 <sup>a</sup>  | 0.44                 |
| 4                                     | 0.50 <sup>a</sup>  | 0.45 <sup>a</sup>  | —                            | —                  | 0.50               | 0.48                 |
| 9                                     | 0.54               | 0.56               | 0.62                         | 0.53               | 0.47               | 0.54                 |
| 10                                    | 0.62 <sup>b</sup>  | 0.64 <sup>b</sup>  | 0.72 <sup>b</sup>            | 0.74 <sup>b</sup>  | 0.55 <sup>b</sup>  | 0.65                 |
| 11                                    | 0.65               | 0.66               | 0.66                         | —                  | 0.55               | 0.58                 |
| 13                                    | 0.40 <sup>b</sup>  | 0.40 <sup>b</sup>  | 0.45 <sup>a</sup>            | 0.45 <sup>a</sup>  | 0.40 <sup>b</sup>  | 0.42                 |
| 16                                    | 0.46 <sup>a</sup>  | 0.50 <sup>a</sup>  | 0.47 <sup>a</sup>            | 0.47 <sup>a</sup>  | 0.60 <sup>a</sup>  | 0.50                 |
| 20                                    | 0.60               | 0.52 <sup>a</sup>  | 0.64                         | 0.58               | 0.54               | 0.58                 |
| Mean                                  | 0.52               | 0.53               | 0.58                         | 0.53               | 0.51               | 0.52                 |

<sup>a</sup> Filtrate cloudy.<sup>b</sup> Filtrate very cloudy.

TABLE 2.—*Per cent citrate-insoluble  $P_2O_5$  in standard phosphate samples as determined by washing with different reagents*

| COLLABORATORS                       | AMMONIATED<br>SUPERPHOS-<br>PHATE | CALCINED<br>PHOSPHATE | TRIPLE<br>SUPER-<br>PHOSPHATE | MIXED<br>FERTILIZER<br>(3-10-5) | MIXED<br>FERTILIZER<br>0.676 (6-8-4) | MIXED<br>FERTILIZER<br>(6-9-4) |
|-------------------------------------|-----------------------------------|-----------------------|-------------------------------|---------------------------------|--------------------------------------|--------------------------------|
| <i>5% Ammonium Citrate Solution</i> |                                   |                       |                               |                                 |                                      |                                |
| 1                                   | 3.27                              | 3.30                  | 2.82                          | 4.40                            | 12.65                                | —                              |
| 2                                   | —                                 | 3.14                  | 2.65                          | 4.42                            | 12.60                                | 0.30                           |
| 3                                   | 2.92                              | 3.67                  | 3.25 <sup>a</sup>             | 4.95                            | 12.87                                | —                              |
| 4                                   | —                                 | 3.55                  | 2.25                          | 4.40                            | 12.90                                | 0.60                           |
| 5                                   | —                                 | 3.47                  | 2.45                          | 4.53                            | 12.90                                | —                              |
| 6                                   | 3.12 <sup>a</sup>                 | 3.29                  | 2.67 <sup>a</sup>             | 4.44 <sup>a</sup>               | 12.66 <sup>a</sup>                   | —                              |
| 7                                   | 5.16                              | 3.37                  | 2.83                          | 4.46                            | 13.11                                | —                              |
| 9                                   | 4.47                              | 3.51                  | 2.77 <sup>a</sup>             | 4.67                            | 13.08                                | 0.50                           |
| 8                                   | 3.42                              | 3.27                  | 1.90 <sup>a</sup>             | 4.47                            | 12.74                                | —                              |
| 10                                  | 3.83 <sup>a</sup>                 | 3.39                  | 3.05 <sup>a</sup>             | 4.99                            | 13.53 <sup>a</sup>                   | 0.75 <sup>a</sup>              |
| 11                                  | 3.23                              | 3.30                  | 2.41 <sup>a</sup>             | 4.45                            | 12.60                                | 0.70                           |
| 12                                  | 4.87                              | 4.65                  | 2.81                          | 4.73                            | 13.29                                | —                              |
| 13                                  | —                                 | 3.40                  | 2.65                          | 4.40                            | 13.05                                | 0.60                           |
| 14                                  | 4.25 <sup>a</sup>                 | 3.40                  | 2.28                          | 4.64                            | 13.22 <sup>a</sup>                   | —                              |
| 15                                  | 4.40                              | 3.27                  | 2.87                          | 4.51                            | 12.86                                | —                              |
| 17                                  | 3.27                              | 3.35                  | 2.51 <sup>b</sup>             | 4.65                            | 13.13 <sup>a</sup>                   | —                              |
| 18                                  | —                                 | 3.12                  | 3.20 <sup>b</sup>             | 4.38 <sup>a</sup>               | 12.35 <sup>b</sup>                   | —                              |
| 19                                  | 4.30                              | 3.65                  | 2.95 <sup>a</sup>             | 4.55                            | 12.85 <sup>a</sup>                   | —                              |
| 20                                  | —                                 | —                     | 2.49                          | 4.45                            | —                                    | —                              |
| 21                                  | 4.94                              | 3.67                  | 2.77                          | 4.60                            | 12.93                                | —                              |
| Mean                                | 3.96                              | 3.46                  | 2.68                          | 4.55                            | 12.91                                | 0.58                           |
| <i>5% Ammonium Nitrate Solution</i> |                                   |                       |                               |                                 |                                      |                                |
| 1                                   | 4.57                              | 3.37                  | 2.85                          | 4.42                            | 12.67                                | —                              |
| 2                                   | —                                 | 3.26                  | 2.87                          | 4.41                            | 12.76                                | 0.35                           |
| 3                                   | 5.08                              | 3.68                  | 3.42                          | 5.03                            | 12.95                                | —                              |
| 4                                   | —                                 | 3.40                  | 2.85                          | 4.45                            | 13.55                                | 0.80                           |
| 5                                   | —                                 | 3.32                  | 2.85 <sup>a</sup>             | 4.47                            | 12.53                                | —                              |
| 6                                   | 4.54                              | 3.39                  | 3.04                          | 4.57                            | 13.00                                | —                              |
| 7                                   | 5.60                              | 3.40                  | 2.92                          | 4.49                            | 13.05                                | —                              |
| 9                                   | 4.40                              | 3.45                  | 3.14                          | 4.74                            | 13.19                                | 0.63                           |
| 8                                   | 4.15                              | 3.37                  | 2.70                          | 4.27                            | 12.81                                | —                              |
| 10                                  | 5.17                              | 3.54                  | 3.15                          | 5.07                            | 13.78                                | 0.85                           |
| 11                                  | 3.85                              | 3.30                  | 2.90                          | 4.47                            | 12.78                                | 0.70                           |
| 12                                  | 5.85                              | 4.17                  | 3.23                          | 4.83                            | 13.41                                | —                              |
| 13                                  | —                                 | 3.50                  | 2.65                          | 4.50                            | 13.35                                | 0.75                           |
| 14                                  | 5.22 <sup>a</sup>                 | 3.53 <sup>a</sup>     | 3.08 <sup>a</sup>             | 4.70                            | 13.43 <sup>a</sup>                   | —                              |
| 15                                  | 5.20                              | 3.33                  | 2.87                          | 4.45                            | 12.89                                | —                              |
| 16                                  | 5.55                              | 3.82                  | 2.89                          | 4.61                            | 13.55                                | 0.70                           |
| 17                                  | 4.36                              | 3.44                  | 3.05                          | 4.64                            | 13.30                                | —                              |
| 18                                  | —                                 | 3.28                  | 2.85 <sup>b</sup>             | 4.57 <sup>a</sup>               | 12.70 <sup>b</sup>                   | —                              |

<sup>a</sup> Filtrate cloudy.<sup>b</sup> Filtrate very cloudy.

TABLE 2.—*Per cent citrate-insoluble  $P_2O_5$  in standard phosphate samples as determined by washing with different reagents (continued)*

| COLLABORATORS                 | AMMONIATED<br>SUPERPHOS-<br>PHATE | CALCINED<br>PHOSPHATE | TRIPLE<br>SUPER-<br>PHOSPHATE | MIXED<br>FERTILIZER<br>(3-10-5) | MIXED<br>FERTILIZER<br>0.676 (6-8-4) | MIXED<br>FERTILIZER<br>(6-9-4) |
|-------------------------------|-----------------------------------|-----------------------|-------------------------------|---------------------------------|--------------------------------------|--------------------------------|
| 19                            | 4.50                              | 3.65                  | 3.00 <sup>a</sup>             | 4.60                            | 12.85 <sup>a</sup>                   | —                              |
| 20                            | —                                 | —                     | 2.29                          | 4.50                            | —                                    | —                              |
| 21                            | 5.54                              | 3.68                  | 3.06                          | 4.59                            | 13.00                                | —                              |
| Mean                          | 4.91                              | 3.49                  | 2.94                          | 4.59                            | 13.08                                | 0.68                           |
| <i>Water at 65° C.</i>        |                                   |                       |                               |                                 |                                      |                                |
| Mean of results<br>in Table 1 | 5.04                              | 3.51                  | 2.56                          | 4.52                            | 12.89                                | 0.52                           |

## COMMENTS OF COLLABORATORS

C. A. Butt.—Noting that the calcined phosphate sample gave an alkaline reaction to the citrate solution, determinations were made of the citrate-insoluble  $P_2O_5$  in the sample with citrate solutions of varying pH. The results obtained are as follows:

| pH OF CITRATE<br>SOLUTION | pH OF CITRATE SOLUTION<br>AFTER DIGESTION | CITRATE-INSOLUBLE<br>$P_2O_5$ FOUND |
|---------------------------|---|-------------------------------------|
|                           |   | <i>per cent</i>                     |
| 5.0                       | 5.1                                       | 2.38                                |
| 6.0                       | 6.7                                       | 3.36                                |
| 7.0                       | 8.0                                       | 3.66                                |
| 8.0                       | 8.1                                       | 4.96                                |

O. W. Ford.—The S and S filters Nos. 589 and 602 are really too slow to permit thorough washing of the insoluble residues. The Shimer filter is very rapid, but it is hard to obtain a uniform pad to hold back the insoluble material in the citrate solution. The present size of the Shimer filter is too small. It would be satisfactory for this work if it were larger and could be padded uniformly.

W. H. MacIntire and L. J. Hardin.—We consider the Shimer filter preferable for several reasons. We never have any trouble with a pad that is  $\frac{1}{4}$ – $\frac{1}{2}$  inch thick when compacted by suction. Use is made of a rubber stopper fastened to a glass rod to compact the filter so that the upper surface is sufficiently compacted to resist disruption when the hot solution is poured directly on the surface of the filter. A filter pad of even twice the thickness we ordinarily use may be washed efficiently with no appreciable retardation in the speed of the filtration. In washing the residue, the wash solution is directed against the side of the filter until it reaches a depth of about one-half inch within the tube. When washing is completed it is easy to push out the hard rubber disk and the tightly-fitting pad and thus wipe cleanly the sides of the filter tube.

## INTERPRETATION OF RESULTS

The time reported by the collaborators for making the filtrations and washings varied greatly, but most agreed in arranging the filters in the following descending order as regards speed of filtering: Shimer filter,

Whatman filter No. 2, Whatman filter No. 5, S and S filter No. 589, and S and S filter No. 602.

The means of the results reported by the collaborators (Table 1) for the different filters are in fairly good agreement, but the results as a whole show (Table 3) that the Shimer filter gives slightly lower values than any of the other filters and S and S filter No. 602 the highest values. In fact, the average value for citrate-insoluble  $P_2O_5$  in the samples as a whole increases as the speed of the filter decreases.

Tables 1 and 2 show that the clearest filtrates were obtained when the washings were made with a 5 per cent ammonium nitrate solution and the most cloudy when the washings were made with water and the use of the Shimer filter. Washing with 5 per cent ammonium nitrate solution also gave a slightly higher result on an average (Table 3) for all samples

TABLE 3.—Average citrate-insoluble  $P_2O_5$  content of all samples as determined by different washing reagents

| 5%<br>NH <sub>4</sub><br>CITRATE SOLN | WATER AT 65° C.   |               |       |               | 5%<br>NH <sub>4</sub> NO <sub>3</sub><br>SOLN |         |
|---------------------------------------|-------------------|---------------|-------|---------------|---|---------|
|                                       | SHIMMER<br>FILTER | WHATMAN PAPER |       | S AND S PAPER |   |         |
|                                       |                   | NO. 2         | NO. 5 | NO. 589       |   | NO. 602 |
|                                       |                   |               |       |               |   |         |
| 4.69                                  | 4.76              | 4.83          | 4.86  | 4.90          | 4.90  | 4.95    |

than did washing with water through any filter. High results with a very slow filter are most likely to arise from inefficient washing and low results from the passage of undissolved material through the filter or from the solvent action of the washing reagent. Most of the collaborators used the more rapid filters (Shimer, Whatman No. 2 and No. 5) when washing with the ammonium nitrate solution. It would seem, therefore, that as a rule the flocculating action of this washing reagent more than offsets its solvent action and that the mean of the results obtained with it most nearly represents the true values for citrate-insoluble  $P_2O_5$  in the samples.

The use of ammonium citrate solution as a washing reagent gave low results, and this was particularly noticeable with Sample 1 (ammoniated superphosphate). The principal phosphatic component of this material, according to Keenen,<sup>1</sup> is tricalcium phosphate, which has been shown by Ross, Jacob, and Beeson<sup>2</sup> and others to be somewhat soluble in neutral ammonium citrate solution. An additional treatment with ammonium citrate, therefore, should have a marked effect in decreasing the citrate-insoluble  $P_2O_5$  in the sample, and this was found to be true by all the collaborators. The phosphatic component of the citrate-insoluble portion of the other samples is mainly raw phosphate rock. This material differs from tricalcium phosphate in that it has a relatively low solubility in

<sup>1</sup> *Ind. Eng. Chem.*, 22, 1378 (1930).

<sup>2</sup> *This Journal*, 15, 227 (1932).

neutral ammonium citrate solution, as shown by Ross, Jacob, and Beeson.<sup>1</sup> This reagent would therefore be expected to have little solvent action on residues that consist largely of undecomposed rock, and this was also corroborated by the collaborators.

Whatman filter paper No. 5 is somewhat less retentive than are S and S filters Nos. 589 and 602, but the differences are not great, and for general control work the greater speed of the Whatman paper would seem to justify its use in place of the slower filter papers.

Many of the collaborators expressed a preference for the Shimer filter, and some of the reports submitted indicate that this filter will give satisfactory results providing the filter pulp mat is made sufficiently thick and compact as directed by MacIntire and Hardin.<sup>2</sup> It would seem, too, that the convenience of the Shimer filter in filtering citrate-insoluble residues might be increased by flaring the upper portion of the tube.

Triple superphosphate Sample 3 was diluted 4.9 fold in the preparation of the mixed fertilizer Sample 6. The results submitted by the collaborators also show that the citrate-insoluble  $P_2O_5$  in Sample 3 is 4.9 times greater than in Sample 6, indicating that dilution with the other materials used in the preparation of Sample 6 has little or no effect on the citrate solubility of the  $P_2O_5$  in triple superphosphate. Dilution of a phosphatic material prior to a citrate-insoluble determination is basically equivalent to an increase in the volume of the citrate solution with which the phosphatic material is treated. While this has little effect on the citrate-solubility of triple superphosphate, or other material containing undecomposed rock, it does increase the citrate-solubility of an ammoniated superphosphate, as already explained.

One of the samples used in this collaborative study was a new phosphatic material called calcined phosphate. Jacob, Rader and Tremearne<sup>3</sup> show that this material has more properties in common with basic slag than with superphosphate, and it is therefore possible that 2 per cent citric acid solution might be better adapted for determining the fertilizer efficiency of this material than neutral ammonium citrate solution.

#### RECOMMENDATIONS<sup>4</sup>

It is recommended—

1. That the words "quick acting filter," *Methods of Analysis, A.O.A.C.*, sec. 14(a), p. 18, line 3, be changed to read "Whatman filter paper No. 5 or other filter paper of equal speed and retentiveness. It is recommended that filtrations be made with suction with the use of a Büchner funnel or ordinary glass funnel with a platinum or other cone"; and that the next sentence ending with " $H_2O$ " be followed by the sentence—"If

<sup>1</sup> Loc. cit.

<sup>2</sup> Loc. cit.

<sup>3</sup> *This Journal*, 19, No. 3 (1936).

<sup>4</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 49 (1936).

the sample is one that gives a cloudy filtrate, wash with a 5 per cent solution of ammonium nitrate" (first action, official).

2. That a further study be made of the application of filter pulp in the Shimer filter to the filtration of citrate-insoluble residues.

3. That a collaborative study be made of methods for determining the availability of calcined phosphate.

### REPORT ON NITROGEN\*

By A. L. PRINCE (Agricultural Experiment Station,  
New Brunswick, N. J.), *Associate Referee*

A new procedure for the determination of water-insoluble nitrogen in cyanamid was adopted last year as a tentative method.<sup>1</sup> The essential modification of this method over the regular method<sup>2</sup> consisted in grinding the sample in water prior to thorough washing on the filter paper. It was also recommended at the last meeting<sup>3</sup> that this procedure be subjected to a collaborative study.

Before carrying out this collaborative study, the associate referee experimented further on various means of wetting the cyanamid previous to washing with water on the filter paper. This work was carried on with the idea of simplifying the tentative method, if possible. Moistening the material with alcohol, or with ether, before washing with water on the filter paper was tried. Mixing equal amounts of ground rock phosphate with the cyanamid prior to washing, in order to change the physical structure of the mixture, was also attempted. The results of this preliminary work indicate that the rock phosphate mixture would yield results quite close to the tentative method providing 350 cc. of wash water were used. The alcohol treatment also gave fair results, but when ether was used to moisten the cyanamid very high results were obtained for water-insoluble nitrogen. In all cases, it seemed essential to wash the material with at least 350 cc. of water.

Accordingly, five series of experiments were planned for a collaborative study on two types of material, granular cyanamid and pulverized cyanamid. The first series followed the regular official method for mixed fertilizers, which consists in placing the sample on a filter paper and washing to 200 cc. with water. In the second series the washing was continued to 350 cc., while in the third series 2 grams of ground phosphate rock was mixed with 2 grams of the sample, transferred to the filter paper,

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\* Journal series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

<sup>1</sup> *This Journal*, 18, 220 (1935).

<sup>2</sup> *Methods of Analysis, A.O.A.C.*, 1930, 22, (32b).

<sup>3</sup> *This Journal*, 18, 62 (1936).

and washed to 350 cc. The new tentative method was applied in the fourth series. In the fifth series, the sample of cyanamid was first moistened with 7 cc. of alcohol, and the washing was continued to 350 cc. with water.

The detailed instructions sent out to the collaborators were as follows:

#### INSTRUCTIONS TO COLLABORATORS

Two samples of cyanamid will be submitted, granular and pulverized. The sample of granular cyanamid should be ground as rapidly as possible in a mortar and transferred to the bottle.

In the following experiments, proper washing of the cyanamid on the filter paper is important. Be sure to allow complete drainage before adding more water to the filter paper. Use Whatman No. 2, 11 cm. filter paper for all filtrations.

*Series I.*—Run each sample in triplicate according to (b), par. 32, p. 22, *Methods of Analysis*, A.O.A.C., 1930.

*Series II.*—Repeat *Series I*, except to continue the washing to 350 cc. instead of 200 cc.

*Series III.*—Add 2 grams of ground rock phosphate to the 2 gram sample of cyanamid, mix thoroughly, transfer to the filter, and wash to 350 cc. From this point continue as directed in *Series I*. Run in triplicate.

*Series IV.*—Run each sample in triplicate according to the method adopted as tentative in 1934.

*Series V.*—Weigh out a 2 gram sample and transfer to the filter paper; wash with about 7 cc. of ethyl alcohol and then with 350 cc. of distilled water. Determine the nitrogen in the residue as usual.

The collaborators were the following:

1. A. H. Allen, Virginia-Carolina Chemical Corp., Richmond, Va.
2. R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
3. M. H. Thornton, Agr. Expt. Sta., Purdue Univ., Lafayette, Ind.
4. M. P. Etheredge, Mississippi State Chem. Lab., A. & M. College, Miss.
5. H. C. Batton, Swift and Company, Baltimore, Md.
6. R. C. Koch, Swift and Company, Hammond, Ind.
7. E. K. Rist, Bureau of Chemistry and Soils, Washington, D.C.
8. S. E. Asbury, Agr. Expt. Station, College Station, Texas.
9. W. H. Garman, Agr. Expt. Station, College Station, Texas.
10. A. N. Lineweaver, Royster Guano Company, Norfolk, Va.
11. A. L. Prince, Associate Referee.
12. A. O. Olson, Dairy and Food Department, St. Paul, Minn.
13. W. L. Adams, Agr. Expt. Station, Kingston, R.I.
14. L. J. Hardin, Univ. of Tennessee Agr. Expt. Sta., Knoxville, Tenn.
15. E. F. Boyce, Agr. Expt. Station, Burlington, Vt.
16. H. R. DeRose, Agr. Expt. Station, Amherst, Mass.

Of the 16 collaborators submitting reports, 5 are from fertilizer companies and 11 are from Experiment Stations or similar institutions.

The data from the collaborative study are compiled in Table 1 for the granular cyanamid and in Table 2 for the pulverized cyanamid. Before discussing the results, comments by several of the collaborators regarding their results should be recorded.

TABLE 1.—*Results of analysis of granular cyanamid for water-insoluble nitrogen*  
(Each result represents an average of three determinations)

| COLLABORATORS | SERIES I        | SERIES II       | SERIES III      | SERIES IV       | SERIES V        |
|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|               | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1             | 2.44            | 0.70            | 0.78            | 0.62            | 0.53            |
| 2             | 1.44            | 0.66            | 0.69            | 0.63            | 1.38            |
| 3             | 0.62            | 0.73            | 0.54            | 0.55            | 0.70            |
| 4             | 1.08            | 0.60            | 0.44            | 0.34            | 0.46            |
| 5             | 0.77            | 0.70            | 0.75            | 0.70            | 0.74            |
| 6             | 0.99            | 0.74            | 0.78            | 0.69            | 1.68            |
| 7             | 0.71            | 0.52            | 0.64            | 0.55            | 0.53            |
| 8             | 0.44            | 0.38            | 0.61            | —               | —               |
| 9             | 0.83            | 0.64            | 1.44            | 0.53            | —               |
| 10            | 4.27            | 1.94            | 0.80            | 0.81            | 0.57            |
| 11            | 2.40            | 0.75            | 0.71            | 0.64            | 1.80            |
| 12            | 1.38            | 1.37            | 2.25*           | 1.73*           | 1.17            |
| 13            | 0.65            | 0.80            | 0.74            | 0.47            | 0.72            |
| 14            | 0.67            | 0.59            | 0.66            | 0.60            | 0.55            |
| 15            | 0.84            | 0.59            | 0.71            | 0.45            | 0.68            |
| 16            | 6.00            | 1.18            | 1.73*           | 1.31*           | 2.21*           |
| Average       | 1.60            | 0.81            | 0.74            | 0.58            | 0.88            |

\* Omitted from average.

TABLE 2.—*Results of analysis of pulverized cyanamid for water-insoluble nitrogen*  
(Each result represents an average of three determinations)

| COLLABORATORS | SERIES I        | SERIES II       | SERIES III      | SERIES IV       | SERIES V        |
|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|               | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| 1             | 2.18            | 0.92            | 0.75            | 0.83            | 0.70            |
| 2             | 6.91            | 1.14            | 0.79            | 0.92            | 1.37            |
| 3             | 0.73            | 0.78            | 0.64            | 0.69            | 0.79            |
| 4             | 1.20            | 1.00            | 0.57            | 0.43            | 0.46            |
| 5             | 0.92            | 0.80            | 0.86            | 0.78            | 1.15            |
| 6             | 1.81            | 1.22            | 0.89            | 0.86            | 1.43            |
| 7             | 0.74            | 0.53            | 0.39            | 0.39            | 1.32            |
| 8             | 0.59            | 0.46            | 0.68            | —               | —               |
| 9             | 1.43            | 0.77            | 1.10            | 0.62            | —               |
| 10            | 5.69            | 4.04            | 2.60*           | 1.13            | 0.67            |
| 11            | 4.10            | 0.83            | 0.94            | 0.79            | 1.09            |
| 12            | 9.55*           | 2.08            | 2.05*           | 2.39*           | 1.87            |
| 13            | 6.54            | 0.89            | 0.84            | 0.55            | 0.74            |
| 14            | 1.24            | 1.45            | 0.78            | 0.80            | 0.74            |
| 15            | 0.71            | 0.64            | 0.67            | 0.50            | 0.65            |
| 16            | 6.12            | 1.68            | 2.08*           | 1.33*           | 2.48*           |
| Average       | 2.72            | 1.20            | 0.76            | 0.71            | 1.00            |

\* Omitted from average.



## COMMENTS BY COLLABORATORS

No. 1. Series I was not at all satisfactory. It did not seem possible to get concordant results by washing with the amount of water prescribed. The remaining series worked out very well.

No. 4. Series III and V are in fair agreement, but the results on Series IV appear to be the most reliable.

No. 8.—The water was not wetting the cyanamid very well, therefore I mixed the material with the water with a stirring rod until the water had wet the sample.

No. 10.—The question of washing out all of the nitrogen in a fertilizer sample is quite an important one, and different chemists, using different filter papers and using different lengths of time for filtering, will get varying results. Of course, the coarser the paper, the quicker the material goes through, and the shorter time it stays in contact with the water, the less nitrogen dissolves out. I am convinced that in order to get uniform results it is necessary to use uniform papers and about the same length of time to wash out the sample. I believe that when any washing of a sample is to be done with water, if the sample is greasy or does not wet easily, it should always be wet with alcohol, and that this practice should be universal. You will observe that the results obtained vary considerably with any one method, but that the variations are very much greater where no alcohol is used than where it is used.

No. 13.—In all cases with the pulverized cyanamid I noticed a great tendency on the part of this material to form lumps, and therefore to shed water. These lumps were, however, broken up as much as possible with small stirring rods.

No. 14.—The preferred procedure seems to be the preliminary washing with alcohol. This, of course, is not so essential in the granular product as it is in the pulverized material. Consistently, however, and for both products, it seems that the washing with alcohol gives better concordance.

In addition to the five series outlined by the associate referee, Collaborator No. 15 tried out another modification. In his method the material is wet with 1 cc. of ethyl alcohol, and then 50 cc. of water is added. After being pulverized and mixed several times with a flattened stirring rod, the mixture is allowed to stand overnight. It is then transferred to a 25 cc. Gooch crucible with a perforated removable bottom and fitted with an asbestos mat, filtered by gentle suction, and washed to 350 cc. This collaborator points out that the advantage of his modification is more complete solution, especially in the case of the granulated form. His general comments were as follows:

No. 15.—From the results as I have studied them, it is apparent that there is quite a difference in the percentage of nitrogen, and I feel that *Series IV* gives the best results. Perhaps you have tried out our modifications. *Series IV* can be run more quickly, since it is not necessary to let stand overnight, but we have found that if we make the weighings before leaving the laboratories at night they can be taken up immediately in the morning for filtration and digestion.

It might be pointed out here that this collaborator obtained 0.45 per cent water-insoluble nitrogen on the granular cyanamid by *Series IV*, and 0.43 per cent by his modification. With pulverized cyanamid the results were 0.50 and 0.45, respectively.

## DISCUSSION

Under Series I, Tables 1 and 2, the wide variation is quite apparent, especially on the sample of pulverized cyanamid. Such results were expected, and this procedure was introduced merely as a means of comparison. The difficulty of wetting the material, together with insufficient washing (200 cc.), accounts for the variation in results. Furthermore, a few of the collaborators, failing to obtain fair results with this method, modified the official procedure by mixing the material in water with a stirring rod prior to washing. With the granular cyanamid in Series I, the results varied from about 0.5 to 2.5 per cent water-insoluble nitrogen; with the pulverized cyanamid, the variation was from about 0.5 to nearly 7 per cent.

In Series II, Tables 1 and 2, are given the results of continued washing of the cyanamid to a volume of 350 cc. A marked improvement is seen in these results over those in Series I, but the percentages are still quite variable and irregular, especially with the pulverized cyanamid. Here the variation with both materials runs from about 0.4 per cent to over 2 per cent.

In Series III, where rock phosphate was incorporated with the sample, the collaborative results appear much more consistent. The average per cent water-insoluble nitrogen on the granular cyanamid was 0.74, with the extremes running 0.44–1.44 per cent. The average per cent water-insoluble nitrogen with pulverized cyanamid was 0.76, the extremes being 0.39 and 1.10 per cent. This method has an advantage over the method in Series IV, in regard to simplicity, but the collaborative results on Series IV were the most constant of all.

In Series 4 of both tables the results by the tentative method adopted last year are recorded. It will be noted that the average results for water-insoluble nitrogen on either granular or pulverized cyanamid are lower than those on any of the other series. The average for the granular cyanamid was 0.58 and 0.71 per cent for the pulverized material. More important, however, is the fact that the individual collaborative results are more nearly alike by this method than by the other methods. This appears to be the most significant factor in deciding which method is best for official use. Although some chemists may obtain just as good results with another procedure, using their own technic, nevertheless the procedure by which the majority of chemists obtain similar results should be the one chosen as the official method.

This statement can well apply to the results obtained in Series V. A number of the collaborators obtained excellent results in this series when alcohol was used to moisten the material prior to washing with water. However, it is evident that they are quite irregular, varying from 0.40 to about 1.90 per cent for both granular and pulverized cyanamid.

The essential point in connection with the analysis of cyanamid for water-insoluble nitrogen is the complete reaction of cyanamid with water, and not merely a superficial washing on the filter paper. The procedure as outlined in Series 4, namely, the tentative method, enables such a reaction to take place, and fairly concordant results have been obtained by such a procedure.

It has been pointed out that certain fertilizer materials, other than cyanamid, moisten with difficulty on the filter paper when being extracted for water-soluble nitrogen. This is usually due to the greasy character of the fertilizer. Moistening the material with a few cc. of alcohol prior to washing with alcohol will no doubt help to overcome this difficulty. An additional statement, allowing the use of alcohol in cases where the fertilizer does not wet easily, might be desirable if inserted under (b) par. 32, p. 22 of the 1930 edition of *Methods of Analysis*. To be consistent, similar additions should be made in the proper places under paragraphs 36, 37, and 39 of the Nitrogen Activity Methods.

#### RECOMMENDATIONS

It is recommended—

(1) That the method adopted last year as tentative for the determination of water-insoluble nitrogen in cyanamid be adopted as official (first action).

(2) That the following statement be inserted after the first sentence under (b), par. 32, p. 22 of the 1930 edition of *Methods of Analysis*: "In case the fertilizer mixture is greasy or does not wet easily, moisten the dry sample with 7 cc. of ethyl alcohol and continue the washing to nearly 200 cc." (first action).

(3) That the following additions be made to the Nitrogen Activity Methods, pp. 23 and 24, 1930 edition of *Methods of Analysis*, official, first action.

P. 23, 36, line 1: After "filter paper," insert "wet with alcohol"; 37, line 2: After "filter paper," insert "wet with alcohol."

P. 24, 39, line 2: After "filter paper," add "wet with alcohol"; line 5: After "small beaker," add "wet with alcohol."

Under (b), line 3: After "filter paper," add "wet with alcohol."

#### REPORT ON ACID-FORMING FERTILIZERS\*

By JOHN B. SMITH (Agricultural Experiment Station,  
Kingston, R. I.), *Associate Referee*

The Pierre method<sup>1</sup> for determining whether a fertilizer is acid-forming or non-acid forming under cropping conditions was discussed last year.<sup>2</sup>

\* Published by permission of the Director of Research as Contribution No. 489 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 229-234 (1933).

<sup>2</sup> *This Journal*, 18, 221-237 (1935).

No action was taken because of some doubt concerning the validity of the acid value assigned to nitrogen, and the justification for subtracting from the apparent basicity of citrate-insoluble  $P_2O_5$  two-thirds of the calcium carbonate equivalent, on the grounds that phosphate rock is not decomposed in the soil at a rate to prevent an increase in acidity from acid-forming fertilizers.

A sub-committee of the Committee on Fertilizers for the American Society of Agronomy was appointed to study these questions. L. G. Willis, the chairman of this committee, has prepared a report to be presented at this meeting<sup>1</sup> discussing the work of the committee, and the recommendations from their findings. The recommendations justify the acceptance of the Pierre method, with the correction for citrate-insoluble  $P_2O_5$ , as a practical basis for distinguishing between acid-forming and base-forming fertilizers.

This year, as last, E. W. Magruder gave valuable aid by sending two samples prepared in this laboratory to the analysts who receive his check samples each month. The method submitted is that published in the report last year and cited previously. Formulas were as follows:

| <i>Sample 8, 4-8-8 Grade</i> |                     | <i>Sample 9, 5-8-7 Grade</i> |                    |
|------------------------------|---------------------|------------------------------|--------------------|
| <i>lbs.</i>                  |                     | <i>lbs.</i>                  |                    |
| 170                          | Calnitro            | 120                          | Nitrate of soda    |
| 220                          | Sulfate of ammonia  | 195                          | Sulfate of ammonia |
| 870                          | Superphosphate      | 45                           | Cyanamid           |
| 270                          | Muriate of potash   | 22                           | Urea               |
| 80                           | Dolomite            | 300                          | Cottonseed meal    |
| 390                          | Organic conditioner | 970                          | Superphosphate     |
|                              |                     | 276                          | Muriate of potash  |
|                              |                     | 72                           | Peat               |

The results, recorded in Table 1, show considerable variability, but a large proportion falls within satisfactory limits. The variations result from differences in the basicity of the ash. Collaborative results have now been published for 21 samples. The average deviation has been 13 pounds of calcium carbonate equivalent per ton, and experienced workers will probably obtain results within this limit. The end point of the titration is troublesome. Pierre, in a private communication, recommends titration on a white porcelain plate while looking through the solution toward an artificial light placed at the proper angle. It is important to stop at the first change in color. Weaker concentrations of the standard sodium hydroxide have been suggested, but in this case each drop causes a smaller change that is more difficult to observe. As now written, each drop of normal sodium hydroxide represents only 5 pounds of calcium carbonate equivalent.

<sup>1</sup> *This Journal*, 19, 309 (1936).

TABLE 1.—*Collaborative results*

| SAMPLE 8, 4-8-8 GRADE            |                             |   |             | SAMPLE 9, 5-8-7 GRADE            |                             |   |             |
|----------------------------------|-----------------------------|---|-------------|----------------------------------|-----------------------------|---|-------------|
| CaCO <sub>3</sub> EQUIVALENT OF— |                             |   |             | CaCO <sub>3</sub> EQUIVALENT OF— |                             |   |             |
| ASH                              | $\frac{1}{2}$ THE N<br>ACID | $\frac{1}{2}$ THE<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub><br>ACID | NET<br>ACID | ASH                              | $\frac{1}{2}$ THE N<br>ACID | $\frac{1}{2}$ THE<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub><br>ACID | NET<br>ACID |
| 38 B                             | 145                         | 4   | 111         | 81                               | 165                         | 12  | 96          |
| 40 B                             | 150                         | 4   | 114         | 70                               | 162                         | 13  | 105         |
| 31 B                             | 150                         | 4   | 123         | 70                               | 164                         | 12  | 106         |
| 15 B                             | 150                         | 4   | 139         | 53                               | 158                         | 8   | 113         |
| 4 B                              | 150                         | 2   | 148         | 56                               | 160                         | 10  | 114         |
| 5 B                              | 151                         | 2   | 148         | 54                               | 160                         | 10  | 116         |
| 4 B                              | 150                         | 6   | 152         | 58                               | 165                         | 11  | 118         |
| 6 B                              | 146                         | 12  | 152         | 56                               | 165                         | 10  | 119         |
| 5 B                              | 154                         | 4   | 153         | 47                               | 160                         | 7   | 120         |
| 1 B                              | 154                         | 2   | 155         | 60                               | 167                         | 14  | 121         |
| 3 A                              | 151                         | 2   | 156         | 50                               | 160                         | 11  | 121         |
| 7 A                              | 148                         | 2   | 157         | 48                               | 163                         | 11  | 126         |
| 4 A                              | 150                         | 3   | 157         | 42                               | 158                         | 10  | 126         |
| 3 A                              | 153                         | 2   | 158         | 35                               | 164                         | 2   | 131         |
| 10 A                             | 146                         | 4   | 160         | 42                               | 161                         | 13  | 132         |
| 10 A                             | 149                         | 2   | 161         | 39                               | 165                         | 9   | 135         |
| 7 A                              | 152                         | 4   | 163         | 40                               | 165                         | 12  | 137         |
| 13 A                             | 151                         | 3   | 167         | 40                               | 164                         | 13  | 137         |
| 17 A                             | 149                         | 4   | 170         | 36                               | 166                         | 9   | 139         |
| 21 A                             | 147                         | 2   | 170         | 40                               | 166                         | 14  | 140         |
| 13 A                             | 153                         | 4   | 170         | 35                               | 164                         | 12  | 141         |
| 16 A                             | 154                         | 0   | 170         | 31                               | 161                         | 11  | 141         |
| 21 A                             | 150                         | 2   | 173         | 32                               | 163                         | 11  | 142         |
| 19 A                             | 149                         | 5   | 173         | 30                               | 164                         | 9   | 143         |
| 19 A                             | 154                         | 2   | 175         | 36                               | 165                         | 14  | 143         |
| 22 A                             | 151                         | 2   | 175         | 30                               | 164                         | 10  | 144         |
| 28 A                             | 147                         | 2   | 177         | 34                               | 168                         | 10  | 144         |
| 27 A                             | 146                         | 5   | 178         | 30                               | 165                         | 12  | 147         |
| 25 A                             | 150                         | 4   | 179         | 29                               | 164                         | 13  | 148         |
| 31 A                             | 149                         | 2   | 183         | 25                               | 162                         | 12  | 149         |
| 36 A                             | 148                         | 2   | 186         | 31                               | 171                         | 12  | 152         |
| 39 A                             | 149                         | 4   | 192         | 14                               | 165                         | 3   | 154         |
| 43 A                             | 148                         | 4   | 195         |                                  |                             |   |             |
| 36 A                             | 153                         | 7   | 196         |                                  |                             |   |             |
| 51 A                             | 151                         | 1   | 203         |                                  |                             |   |             |
| Average                          |                             |   | 163         | Average                          |                             |   | 133         |
| Average Deviation                |                             |   | 16          | Average Deviation                |                             |   | 13          |
| Maximum Deviation                |                             |   | 53          | Maximum Deviation                |                             |   | 37          |
| A—Acid                           |                             |   |             |                                  |                             |   |             |
| B—Basic                          |                             |   |             |                                  |                             |   |             |

Originally, the method suggested 100 cc. porcelain beakers for ashing. These are very convenient but not readily available. The Central Scientific Company has undertaken to supply this size in silimanite.

Evidence is accumulating showing the slow reaction rate of dolomite coarser than 20-mesh. Pierre suggests that this coarse material be removed by wet sieving before the sodium-carbonate-sucrose solution is added. This suggestion deserves study next year.

The method is published below to include a few editorial changes suggested during the past year.

#### FERTILIZER REACTION

##### REAGENTS

(a) *Methyl red indicator*.—0.2% solution. Dissolve 1 gram of the dye in 300 cc. of 95% alcohol and dilute to 500 cc. with water.

(b) *Na<sub>2</sub>CO<sub>3</sub>-Sucrose solution*.—Dissolve 106 grams of anhydrous Na<sub>2</sub>CO<sub>3</sub>, or 286 grams of Na<sub>2</sub>CO<sub>3</sub> · 10 H<sub>2</sub>O, and 50 grams of sucrose in water. Make to a volume of 1 liter. Pipet 10 cc. into a 250 cc. Erlenmeyer flask, add 30 cc. of normal HCl solution carefully, and boil gently for a few minutes to remove CO<sub>2</sub>. Titrate with normal NaOH to the first color change of methyl red indicator. The difference between the volumes of standard acid and base used is the blank for the solution.

##### DETERMINATION

If the fertilizer mixture, ground as directed under 2,<sup>1</sup> contains less than 30% as the sum of the percentages of total N, available P<sub>2</sub>O<sub>5</sub>, and water-soluble potash, weigh 1 gram of the mixture into a 100 or 150 cc. porcelain or Pyrex glass beaker. If the sum of these percentages is 30 or more, use 0.5 gram, and for salts of N or K use 0.25 gram. With a pipet or buret add 10 cc. of the Na<sub>2</sub>CO<sub>3</sub>—sucrose solution, and mix thoroughly with the fertilizer, except for unmixed nitrate salts. For these, substitute 0.25 gram of carbon black for the sucrose. Place in a sand bath to the depth of the mixture in the beaker and evaporate to complete dryness. Place the beakers in a furnace heated to approximately 250° C., and raise the temperature gradually to 500–600° C. (dull red). Hold at this temperature for one hour. (It is not necessary that all carbon be removed.) Remove the beakers and allow to cool. Add 50 cc. of water, cover with a watch-glass, and add 30 cc. of normal HCl through the lip of the beaker. After effervescence ceases, place the covered beaker on a hot plate or steam bath and maintain just below the boiling point for one hour. Filter the solution through a disk of filter paper, or a pad of asbestos that has been digested with normal HCl and washed free from acid with water, using a Gooch crucible and suction. Wash with hot water. To the entire clear filtrate, approximately 100 cc., add 10 drops of methyl red indicator, and titrate to the *first* change in color, orange-pink. (In determining this end point a duplicate solution of the fertilizer ash displaying the maximum acid color for this indicator may be used as a comparison to determine the first change. The titration is conveniently carried out on a white porcelain plate, and by using an artificial daylight bulb placed at a convenient angle above and back of the porcelain plate.

Subtract the cc. of normal NaOH used in the titration from the cc. of normal HCl added. From the difference subtract algebraically the blank caused by the Na<sub>2</sub>CO<sub>3</sub>—sucrose solution. For a 1 gram sample multiply the result by 100; 0.5 gram sample, by 200; 0.25 gram sample, by 400. Positive values represent the excess base in the ash in pounds of CaCO<sub>3</sub> equivalent per ton of fertilizer. Negative values represent excess acidity in the same terms.

Determine total nitrogen as directed under 24 or 29. Multiply the percentage

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 1.

of nitrogen by 35.7. This is considered to be the acid-forming power of the nitrogen in terms of pounds of  $\text{CaCO}_3$  equivalent per ton of fertilizer and is given a negative sign in calculating the net acid-base balance.

Determine the citrate-insoluble  $\text{P}_2\text{O}_5$  as directed under 13, 14. The percentage found, multiplied by 28.2, is the alkalinity equivalent to 2 of the 3 Ca atoms of  $\text{Ca}_3(\text{PO}_4)_2$  expressed in terms of pounds of  $\text{CaCO}_3$  equivalent per ton of fertilizer. Correct the net balance for the fertilizer for this basicity, assumed to be relatively inactive in the soil, by giving the value a negative sign.

The algebraic sum of the acid-base balance of the ash and the corrections for nitrogen and citrate-insoluble  $\text{P}_2\text{O}_5$  is the net balance of the fertilizer expressed as pounds of  $\text{CaCO}_3$  equivalent per ton. If negative, the fertilizer is considered acid-forming; if positive, it is considered non-acid forming.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That the modified Pierre method for determining whether a fertilizer is acid-forming or non-acid-forming be adopted as tentative.
- (2) That elimination of water-soluble material coarser than 20-mesh by wet sieving, before the method is applied, be studied next year.
- (3) That the basicity of phosphate rock and other factors that affect the method be studied further.

### REPORT ON MAGNESIUM IN FERTILIZERS\*

By JOHN B. SMITH (Agricultural Experiment Station,  
Kingston, R. I.), *Associate Referee*

This report presents a proposed method for the determination of total magnesium in fertilizer mixtures and data concerning the solubility of various carriers of magnesium in a number of solvents under different conditions. These data should provide a basis for a method for available magnesium as soon as agronomic research defines the portion of the common magnesium compounds that should be included in such a method. This should be possible very soon, for many data are now available and more research is under way.

#### TOTAL MAGNESIUM

After consultation with others familiar with the possibilities and considerable work in the laboratory, the associate referee concluded that the method proposed by Hoffman<sup>2</sup> for the determination of magnesia in phosphate rock seemed best adapted for total magnesium because of its simplicity and accuracy.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 49 (1936).

\* Contribution No. 484 of the Station.

The Associate Referee is indebted to H. D. Haskins and J. W. Kusmeski, of the Massachusetts Agricultural Experiment Station, for generous collaboration on this problem, much of it independent in nature, adding substantially to the scope of the work. Also to E. J. Dessyok, of this laboratory, for critical observation and care in technical details.

<sup>2</sup> *Bur. Standards J. Research*, 9, 487-491 (1932).

The method, changed very little from the original, is given here in detail. It includes dissolving the sample in hydrochloric and nitric acids, as is frequently done for  $P_2O_5$ , further destruction of organic matter and dehydration of silica in sulfuric acid, removal of calcium sulfate by a single precipitation from alcohol, precipitation of magnesium ammonium phosphate in the presence of iron and aluminum, held in solution by citric acid, and a reprecipitation to purify the magnesium ammonium phosphate before the usual ignition. This procedure avoids a double precipitation of calcium and removal of iron and aluminum. Manganese is an interfering element and must be determined in the final precipitate. The latter is a simple colorimetric procedure, and in most cases the amount of potassium permanganate obtained will be too small to require actual determination.

Table 1 shows satisfactory duplication and recovery of magnesium

TABLE 1.—*Recovery of MgO, added as  $MgSO_4$  or dolomite, from fertilizer mixtures*  
(Results expressed as percentages in the entire mixture)

| INGREDIENTS OF FERTILIZER MIXTURES  | MgO<br>ADDED | MgO FOUND |         |         | MgO<br>ADDED | MgO FOUND |         |         |
|---|--------------|-----------|---------|---------|--------------|-----------|---------|---------|
|   |              | ANALYST   | ANALYST | ANALYST |              | ANALYST   | ANALYST | ANALYST |
|   |              | 1         | 2       | 3       |              | 1         | 2       | 3       |
| 3-8-4   |              |           |         |         |              |           |         |         |
| Cottonseed meal, sulfate<br>of ammonia, superphos-<br>phate, muriate of potash,<br>rock phosphate, manganese<br>sulfate | 0.34         |           |         |         | 0.34         |           |         |         |
| Magnesium sulfate   | 2.08         |           |         |         |              |           |         |         |
| Dolomite  |              |           |         |         | 1.87         |           |         |         |
| Entire mixture  | 2.42         | 2.42      | 2.51    | 2.44    | 2.21         | 2.13      | 2.15    | 2.17    |
| 8-16-8  |              |           |         |         |              |           |         |         |
| Cyanamid, urea, basic<br>slag, precipitated bone, mu-<br>riate of potash, quartz sand,<br>manganese sulfate             | 0.24         |           |         |         | 0.22         |           |         |         |
| Magnesium sulfate   | 2.08         |           |         |         |              |           |         |         |
| Dolomite  |              |           |         |         | 1.87         |           |         |         |
| Entire mixture  | 2.32         | 2.18      | 2.17    | 2.40    | 2.09         | 2.12      | 2.10    | 2.10    |
| 4-12-4  |              |           |         |         |              |           |         |         |
| Cottonseed meal, ammo-<br>phos A, muriate of potash,<br>manganese sulfate, quartz<br>sand                               | 0.40         |           |         |         | 0.40         |           |         |         |
| Magnesium sulfate   | 2.08         |           |         |         |              |           |         |         |
| Dolomite  |              |           |         |         | 1.87         |           |         |         |
| Entire mixture  | 2.48         | 2.40      | 2.48    | 2.56    | 2.27         | 2.24      | 2.17    | 2.18    |



oxide added as magnesium sulfate and dolomitic limestone for three mixtures made in this laboratory. Table 2 records comparisons of results by this method and by other common procedures obtained on the same mixtures at the Massachusetts Station under the direction of Haskins and at the Rhode Island Station. Results by the method proposed by Bartlett<sup>1</sup> are included to show that this procedure, which attempts to shorten the standard method for magnesium oxide, is not always satisfactory. The results by the other procedures agree well, although those by the sodium acetate method ran consistently higher than those obtained by the Hoffman method. Haskins states that while the Hoffman method did not appeal at the start, it became the favorite method as the work pro-

TABLE 2.—Results on acid-soluble MgO obtained by Hoffman method compared with those obtained by other accepted methods

(Analyses by J. W. Kuzmeski, Mass. Agr. Exp. Sta., except those labelled "R.I.," which were made by E. J. Deszyck)

|                            | HOFFMAN METHOD |          | A.O.A.C.<br>METHOD<br>FOR LIME | SODIUM<br>ACETATE<br>METHOD | BARTLETT<br>METHOD |
|----------------------------|----------------|----------|--------------------------------|-----------------------------|--------------------|
|                            | per cent       | per cent | per cent                       | per cent                    | per cent           |
| Calcined Kieserite 1       |                | 31.59    | 31.37                          |                             |                    |
| Calcined Kieserite 2       |                | 29.45    | 29.56                          |                             |                    |
| Calcined Kieserite 3       |                | 30.25    | 30.21                          |                             |                    |
| Dolomite 1                 |                | 21.26    | 21.08                          |                             |                    |
| Dolomite 2                 |                | 17.42    | 17.42                          |                             |                    |
| Dolomite 3                 |                | 20.40    | 20.40                          |                             |                    |
| Sulfate of potash-magnesia |                | 9.34     | 9.53                           |                             |                    |
| Serpentine                 |                | 36.26    | 36.15                          |                             |                    |
| Olivine                    |                | 45.82    | 45.93                          |                             |                    |
| Mixed Fertilizers          | R.I.           |          |                                |                             |                    |
| 12-16-12                   | 1.96           | 1.88     |                                | 2.03                        | 1.60               |
| 4-12-4                     | 2.78           | 2.72     |                                | 2.90                        | 2.55               |
| 6-8-6                      | 3.65           | 3.86     |                                | 3.84                        | 3.35               |
| 8-12-20                    | 3.14           | 3.20     |                                | 3.40                        | 3.08               |
| 8-16-16                    | 2.70           | 2.83     |                                | 2.83                        | 2.97               |
| 4-12-4                     | 3.14           | 3.23     |                                | 3.30                        | 3.02               |
| 3-8-5                      | 1.96           | 2.12     |                                | 2.39                        | 1.99               |

ceeded. This has also been the experience at the Rhode Island laboratory.

The evidence seems sufficient to recommend the procedure as a tentative method, to be confirmed by more collaborative work next year. The method follows:

#### TOTAL MAGNESIA

Weigh a 1 gram sample into a 250 cc. beaker, cover, add 10 cc. of HCl and 30 cc. of HNO<sub>3</sub>, and boil gently for 30 minutes. Remove the beaker from the source of

<sup>1</sup> Unpublished.

heat, cool, add 6 cc. of  $\text{H}_2\text{SO}_4$  (1+1), remove the cover, and evaporate until white fumes appear. Cool slightly, wash down the inside surface of the beaker with a jet of water, and again evaporate until fumes of  $\text{H}_2\text{SO}_4$  appear. Cool, add 10 ml. of water, stir thoroughly, and digest on the steam bath for 10 to 15 minutes. Remove from the steam bath, add 100 ml. of 95% alcohol, stir so that the  $\text{CaSO}_4$  is well dispersed throughout the liquid, and allow to stand for 2 hours or longer. Filter by means of suction through a tight plug of filter paper pulp, using a Gooch crucible, and wash five times with 5 cc. portions of 95% alcohol containing 1 cc. of  $\text{H}_2\text{SO}_4$  per 100 cc.

Evaporate the alcoholic filtrate as far as possible on the steam bath. Transfer the solution to a 250 cc. Erlenmeyer flask, dilute to a volume of 75-100 cc., and add 2 grams of citric acid and 10 cc. of a 25% solution of  $(\text{NH}_4)_2\text{HPO}_4$ . Add  $\text{HH}_4\text{OH}$  until the solution is alkaline to litmus and then add 10 cc. in excess. Add 5-10 glass beads, tightly stopper the flask, and shake on a shaking machine for at least 1 hour. Allow to stand in a cool place for 4 hours, or preferably overnight. Filter through a tight paper containing a little paper pulp, and wash with diluted  $\text{NH}_4\text{OH}$  (5+95), containing 50 grams of  $(\text{NH}_4)_2\text{HPO}_4$  per liter, until the precipitate and paper are free from Fe and Al. Pass 25 ml. of hot diluted  $\text{HCl}$  (5+95) through the paper into the flask, transfer the solution to a 250 cc. beaker, and wash the paper and flask thoroughly with more of the diluted acid. To the solution in a volume of 50-75 cc. and containing no glass beads, add 0.5 ml. of a 25% solution of  $(\text{NH}_4)_2\text{HPO}_4$ , cool, and then add  $\text{NH}_4\text{OH}$  slowly and with stirring until the solution is alkaline to litmus. Stir for a few minutes, add 3-4 cc. of  $\text{NH}_4\text{OH}$ , and allow to stand for 4 hours or overnight. Transfer the precipitate to a small filter or filtering crucible and wash with diluted  $\text{NH}_4\text{OH}$  (5+95). Ignite slowly at a temperature below  $900^\circ\text{C}$ . until the carbon is burned (preferably in a muffle furnace with pyrometric control), and then at about  $1,100^\circ\text{C}$ . for 1-2 hours. Cool, and weigh. The residue consists of  $\text{Mg}_2\text{P}_2\text{O}_7$ , and possibly  $\text{Mn}_2\text{P}_2\text{O}_7$  and  $\text{Ca}_3(\text{PO}_4)_2$ . If the alcoholic filtrate was clear, the  $\text{Ca}_3(\text{PO}_4)_2$  will not exceed 0.3 mg. and can be neglected. Make the correction for Mn as follows: Dissolve the residue in 10 cc. of  $\text{H}_2\text{SO}_4$  (1+9), transfer the solution to a 250 cc. Erlenmeyer flask, and add 50 cc. of  $\text{HNO}_3$  (1+3), 2 cc. of sirupy  $\text{H}_3\text{PO}_4$ , sp. gr. 1.7, and 0.2 gram of  $\text{KIO}_4$ . Boil for 15-20 minutes, cool, and dilute to a convenient volume. In another flask containing the same amounts of the reagents treated in a similar way, match the color by adding a standard solution of  $\text{KMnO}_4$ , or compare with a standard solution of  $\text{KMnO}_4$  in a colorimeter. Calculate the manganese as  $\text{Mn}_2\text{P}_2\text{O}_7$  and subtract this weight from the total weight. Regard the difference as  $\text{Mg}_2\text{P}_2\text{O}_7$ , which contains 36.21% of  $\text{MgO}$ .

#### SOLUBILITY OF MAGNESIUM COMPOUNDS

Agronomic data showing the degree of availability of different compounds are being obtained from a number of experiments, supplementing previous research. The solubility of the different carriers in various solvents must be known in order to select a solvent and conditions for measuring a part proportional to the "available" fraction.

The results in Table 3 show the maximum solubility of several salts under the conditions specified for a few of the solvents that have been suggested. Relatively large quantities, representing more than 300 mg. of magnesium oxide, of the different products were placed in the solvents. The temperature of  $25^\circ\text{C}$ . was maintained by a water thermostat, and the stoppered flasks containing the samples were shaken at 10-minute

TABLE 3.—Solubility of magnesium compounds in different solvents (mg. of MgO in 100 cc.)

| SOLVENTS*                             | °C.        | MAGNESIUM                     |                                |  |               |               |               |               |            |         |                    | MAGNESIUM |
|---------------------------------------|------------|-------------------------------|--------------------------------|--|---------------|---------------|---------------|---------------|------------|---------|--------------------|-----------|
|                                       |            | DI-<br>MAGNESIUM<br>PHOSPHATE | TRI-<br>MAGNESIUM<br>PHOSPHATE | AMMONIUM<br>PHOSPHATE<br>WITH 6 MOL.<br>OF WATER | DOLOMITE<br>1 | DOLOMITE<br>2 | DOLOMITE<br>3 | DOLOMITE<br>4 | SERPENTINE | OLIVINE | SILICATE,<br>C. P. |           |
| Water                                 | 25         | 12                            | 3                              | 10   | 2             | 5             | 2             |               | 2          | 1       | †                  |           |
| Water,                                | 60 min.    |                               |                                |  |               |               |               |               |            |         |                    |           |
| Water,                                | 120 min.   | 20                            | 3                              | 7  | 3             | 3             | 2             |               | 2          | 2       | 4                  |           |
| NH <sub>4</sub> Oxalate, <sup>1</sup> | 25         | 51                            | 33                             | 11   | 7             | 11            |               |               |            | †       | 4                  |           |
| "                                     | 25         | 54                            | 56                             | 11   | 10            | 12            |               |               |            | †       | 6                  |           |
| "                                     | 100        | 40                            | 26                             | 46   | 34            | 36            |               |               |            | 7       | 26                 |           |
| "                                     | 100        | 32                            | 24                             | 30   | 22            | 42            |               |               |            | 16      | 29                 |           |
| NH <sub>4</sub> Citrate <sup>2</sup>  | 25         | *VS                           | 262                            | 117  | 9             | 20            |               | 12            | 7          | 1       | 17                 |           |
| "                                     | 25         | VS                            | 265                            | 117  | 13            | 39            |               | 35            | 20         | 3       | 33                 |           |
| "                                     | 100        | VS                            | VS                             | VS   | 214           | 286           |               | 265           | 123        | 37      | 57                 |           |
| "                                     | 100        | VS                            | VS                             | VS   | 228           | 274           |               | 286           | 194        | 57      | 61                 |           |
| NH <sub>4</sub> Cl <sup>3</sup>       | 25         | 111                           | 252                            | 43   | 11            | 31            | 12            |               | 7          | 3       | 15                 |           |
| "                                     | 60 min.    |                               |                                |  |               |               |               |               |            |         |                    |           |
| "                                     | 18.5 hours | 182                           | VS                             | 19   | 11            | 17            | 8             |               | 8          | †       | 21                 |           |
| "                                     | 60 min.    | 184                           | VS                             | 67   | 138           | 162           | 93            |               | 39         | 11      | 83                 |           |
| "                                     | 100        | 185                           | VS                             | 113  | 163           | 258           | 195           |               | 37         | 14      | 97                 |           |

\* Primary magnesium phosphate, sulfate of potash magnesia, and kieserite (calined MgSO<sub>4</sub>)—more than 300 mg.

† Less than 1 mg.

<sup>1</sup> 15 cc. saturated NH<sub>4</sub> oxalate and 185 cc. of water, adjusted to pH 5.<sup>2</sup> Neutral sp. gr. 1.09, as for citrate-insoluble P<sub>2</sub>O<sub>5</sub>.<sup>3</sup> A normal solution neutralised to pH 7 with NaOH.

intervals. The higher temperatures were kept at the boiling point, under reflux condensers, and filtered at this temperature. Aliquots were taken from the hot solution, or the filtrates were diluted to prevent crystallization. Probably the longest time periods are too short for equilibrium to be attained. This is certainly true of the carbonate in hot ammonium solutions, for the decomposition could go on until all the ammonia end-product had been expelled from the alkaline solution. The longer periods are as great as would be practicable, however. All data are averages of two or more results. The causes of a few obvious discrepancies have not been determined.

In studying the table, if a decimal point is placed at the left of the right-hand digit, the percentage of magnesium oxide that should be obtained from a 1 gram charge in 100 cc. of solvent is shown. Mixtures containing these products and presenting no ingredient with an ion common to the product should show slightly greater solubility for magnesium.

Since water-soluble compounds are usually considered as available, the water-soluble fraction of magnesium in fertilizers has been guaranteed in several instances, and many agronomists believe that this most active fraction has special value for some conditions. If used as a general method, this solvent would apparently eliminate dolomites, the silicates, and trimagnesium phosphate as available carriers. However useful this might be for a particular case, it is obviously not adapted to a general method. Its greatest usefulness would seem to be the measurement of water-soluble magnesium oxide in ingredients that manufacturers may desire in their formulas.

Ammonium salts are useful because they decompose carbonates in addition to dissolving other compounds. Ammonium oxalate, recommended by Bartlett for use in Maine, has the advantage of precipitating the calcium while magnesium is dissolved at the same time to the extent of the solubility of magnesium oxalate. Apparently, boiling solutions of the concentration used here, and specified by Bartlett, could dissolve all the magnesium likely to be added to fertilizers as dolomite. This solvent is not so well adapted to the analysis of magnesium compounds used as ingredients in fertilizers because the greater concentration of magnesium causes precipitation of magnesium oxalate. The other ammonium salts could be used by varying time, concentration, and temperature for the results desired.

Samples of olivine and serpentine were included to represent natural silicates, presumably low in availability, but these same samples, particularly the serpentine, have since proved satisfactory sources of magnesium in a pot test. Their position with respect to other carriers needs more study.

Haskins supplied important information showing the solubility of magnesium compounds (Table 4). All the acid-soluble magnesia in the

TABLE 4.—*Solubility of several magnesium compounds in different solvents*  
(per cent of total MgO in the samples)  
(Analyses by J. W. Kuzmeski)

| SOLVENT  | CALCINED<br>KIESER-<br>ITE | CALCINED<br>KIESERITE<br>AND CaCO <sub>3</sub> | "EMJEO" | DOLO-<br>MITTE | DOLO-<br>MITTE | TALC* | SERPEN-<br>TINE† | OLIVINE‡ |
|--|----------------------------|--|---------|----------------|----------------|-------|------------------|----------|
| Hot water  |                            |  |         |                |                |       |                  |          |
| 1 g. in 250 cc   | 99.6                       | 99.4   | 98.1    | —              | —              | —     | T                | T        |
| Cold water   |                            |  |         |                |                |       |                  |          |
| 1 g. in 250 cc.  | 99.0                       | 99.8   | 98.4    | —              | —              | —     | T                | T        |
| 2% citric acid   |                            |  |         |                |                |       |                  |          |
| 2 g. in 100 cc. 1 hr. on steam bath. Filtered and made to 250 cc.  | —                          | —  | —       | 33             | 34             | 12    | 25               | —        |
| 2% citric acid   |                            |  |         |                |                |       |                  |          |
| 1 g. in 100 cc treated as above. Less acid neutralized   | —                          | —  | —       | 60             | 62             | 17    | 30               | —        |
| Neutral NH <sub>4</sub> citrate, sp. gr. 1.09  | —                          | —  | —       | 21             | 25             | 4     | 17               | —        |
| 1% HCl   | —                          | —  | —       | —              | —              | 27    | —                | —        |
| Neutral NH <sub>4</sub> oxalate, 0.5 g. in 150 cc. + 15 cc. sat'd NH <sub>4</sub> oxalate soln, neutralized with HCl. Boiled 10 min. | —                          | —  | —       | —              | —              | 17    | —                | —        |
| 1% HCl + 5 g. dry NH <sub>4</sub> Cl   | —                          | —  | —       | —              | —              | 40    | —                | —        |
| 5% NH <sub>4</sub> Cl, 2 g. in 100 cc., 30 min., 100° C.   | —                          | —  | —       | —              | —              | 4     | —                | —        |

\* Finely ground Total MgO by fusion 32.65%.

† Passed 0.5 mm sieve Acid-soluble MgO, serpentine 36.22%, olivine 45.86%

‡ Trace.

commercial calcined magnesium sulfate, kieserite or "Emjeo," is also soluble in hot or cold water, if proper conditions are maintained. A 2 gram charge of dolomite neutralized 100 cc. of 2 per cent citric acid, allowing a greater proportionate amount to be dissolved from 1 gram. A solution of neutral ammonium citrate dissolved only  $\frac{1}{2}$  of the magnesia in dolomite. The magnesia in talc was appreciably soluble in citric acid, 1 per cent hydrochloric acid, neutral ammonium oxalate, and a solution of 1 per cent hydrochloric acid with 5 grams of ammonium chloride, but much less soluble in neutral ammonium citrate and 5 per cent ammonium chloride. Like serpentine, it was less soluble than dolomite. Of the solvents and conditions tried, the citric acid best approximates the probable available fraction of magnesia.

# SOLUBILITY OF MAGNESIUM COMPOUNDS IN FERTILIZER MIXTURES

Chemical reactions in the curing pile and the inter-effects of ingredients on solubility, and on the solvent, make it impossible to predict the actual solubility of compounds in complex mixtures such as fertilizers. Tests under actual working conditions were carried out with samples of commercial fertilizers, kindly furnished by four different companies. These samples represented actual factory conditions, and, as was expected, the mixtures were not as exact as if they had been prepared in the laboratory. On the other hand, the natural conditions of temperature, moisture, and pressure in the pile are important in the reactions of curing, and the samples that were used proved very helpful in this preliminary survey.

TABLE 5.—*Grades and ingredients of fertilizer mixtures from which samples were taken*

| MIXTURES                            | 12-16-12 | 4-12-4(1) | 4-12-4(2) | 6-8-6 | 8-16-16 | 8-16-20* | 7-16-20* | 8-12-20 | 3-8-5 | 8-16-14* | 4-8-10 |
|-------------------------------------|----------|-----------|-----------|-------|---------|----------|----------|---------|-------|----------|--------|
| Sulfate of Ammonia                  | ×        | ×         | ×         | ×     |         |          |          |         |       |          | ×      |
| Ammonium Phosphate                  | ×        |           |           |       | ×       |          |          | ×       |       |          |        |
| Nitrate of Soda, Arcadian Tankage   |          | ×         |           |       |         |          |          |         | ×     |          | ×      |
| NH <sub>4</sub> -NH <sub>4</sub> OH |          | ×         |           |       |         |          |          |         | ×     |          | ×      |
| Treble Superphosphate               |          | ×         | ×         |       | ×       |          |          |         |       |          |        |
| Superphosphate                      | ×        | ×         | ×         | ×     | ×       |          |          | ×       | ×     |          |        |
| Muriate of Potash                   |          | ×         | ×         | ×     | ×       |          |          | ×       | ×     |          | ×      |
| Nitrate of Potash                   | ×        |           |           |       |         | ×        | ×        |         |       |          |        |
| Calnitro, Dolomitic                 |          |           | ×         | ×     | ×       | ×        | ×        | ×       |       | ×        | ×      |
| Sulfate of Potash-                  |          |           |           |       |         |          |          |         |       |          |        |
| Magnesia                            |          |           |           |       |         |          |          | ×       | ×     |          | ×      |
| Calcined Kieserite                  |          |           |           |       | ×       | ×        | ×        |         |       | ×        | ×      |
| Magnesian Limestone                 | ×        | ×         | ×         | ×     |         |          |          |         | ×     | ×        |        |
| Organic Conditioner                 | ×        |           | ×         | ×     | ×       |          |          | ×       |       |          |        |
| Blood                               |          |           |           |       |         |          |          |         | ×     |          |        |
| Castor Meal                         |          |           |           |       |         |          |          |         | ×     |          |        |
| Filler                              |          |           |           |       |         |          |          |         | ×     |          | ×      |
| Ammoniated Superphosphate           |          |           |           |       |         |          |          |         |       |          | ×      |

\* Partial formula, but includes all carriers of mg.

A list of the samples and ingredients is given in Table 5. With three exceptions, the complete formulas were furnished, and in these instances the weights of the magnesium carriers were available. Samples of all ingredients were also supplied. Portions of seven of the samples and of their components were sent to Haskins for collaboration.

Magnesian limestone, calnitro (ammonium nitrate, with dolomitic limestone in these samples), calcined kieserite, and sulfate of potash-

TABLE 6.—*Solubility of MgO in commercial fertilizer mixtures*  
(Results expressed in percentage)

| MgO CALCULATED FROM FORMULA |                                  |      |               |                | MgO DISSOLVED BY VARIOUS SOLVENTS |                      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-----------------------------|----------------------------------|------|---------------|----------------|-----------------------------------|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| FERTILIZER                  | MAG-<br>NESIAN<br>LIME-<br>STONE |      | CAL-<br>NITRO | KIES-<br>ERITE | SUL-<br>FATE                      | A                    |       | B     |       | C     | D     | E     | F     | G     | H     | I     | J     | K     | L     | M     | N     |       |
|                             |                                  |      |               |                |                                   | OF<br>POTASH<br>MAG. | MASS. | R. I. | MASS. | R. I. | MASS. | MASS. | MASS. | MASS. | MASS. | MASS. | R. I. | R. I. | R. I. | R. I. | R. I. |       |
| 12-16-12                    | 1.61                             |      |               |                |                                   |                      | 1.88  | 1.96  | 0.29  | 0.24  | 0.25  | 0.20  | 0.29  | 1.27  | 0.72  | 0.62  | 0.43  | 0.60  | 0.76  | 1.09  | 0.66  | 0.90  |
| 4-12-4 (1)                  | 3.11                             |      |               |                |                                   |                      | 3.23  | 3.14  | 1.02  | 1.38  | 0.69  |       | 1.07  | 3.21  | 2.58  | 1.25  | 1.07  | 1.42  | 1.67  | 1.95  | 1.64  | 1.87  |
| 4-12-4 (2)                  | 1.54                             | 0.85 |               |                |                                   |                      | 2.72  | 2.78  | 0.50  | 0.67  | 0.63  |       | 0.60  | 1.90  | 1.04  | 0.87  | 0.70  | 0.63  | 1.00  | 1.33  | 0.87  | 1.18  |
| 6-8-6                       | 2.20                             | 1.21 |               |                |                                   |                      | 3.86  | 3.65  | 0.40  | 0.39  | 0.41  |       | 0.45  | 2.67  | 1.49  | 1.03  | 0.64  | 0.69  | 0.96  | 1.39  | 1.10  | 1.66  |
| 8-16-16                     |                                  | 1.23 | 1.58          |                |                                   |                      | 2.83  | 2.70  | 1.36  | 1.60  | 0.66  | 0.67  | 1.83  | 2.54  | 2.26  | 2.26  | 1.70  | 1.79  | 2.11  | 2.25  | 2.06  | 2.25  |
| 8-16-20                     |                                  | 0.68 | 1.64          |                |                                   |                      |       | 2.18  |       | 1.43  |       |       |       |       |       |       |       | 1.69  | 1.81  | 1.91  | 1.74  | 1.80  |
| 7-16-20                     |                                  | 0.62 | 1.48          |                |                                   |                      |       | 1.94  |       | 1.65  |       |       |       |       |       |       |       | 1.74  | 1.93  | 1.95  | 1.81  | 0.15* |
| 8-12-20                     |                                  | 1.18 |               |                |                                   |                      | 1.82  | 3.20  | 3.12  | 0.87  | 1.29  | 0.49  | 1.20  | 3.17  | 2.76  | 2.34  | 2.05  | 2.26  | 2.52  | 2.74  | 2.49  | 2.50  |
| 3-8-5                       | 1.02                             |      |               |                |                                   |                      | 2.14  | 1.96  | 1.05  | 1.56  | 0.82  |       | 1.29  | 2.13  |       | 1.68  | 1.51  | 1.52  | 1.64  | 1.74  | 1.62  | 1.75  |
| 8-16-14                     | 1.39                             | 0.62 | 1.32          |                |                                   |                      |       | 3.25  |       | 1.54  |       |       |       |       |       |       |       | 1.51  | 1.89  | 2.07  | 1.68  | 2.12  |
| 4-8-10                      |                                  | 0.17 | 0.80          | 1.41           |                                   |                      |       | 2.44  |       | 2.02  |       |       |       |       |       |       |       | 2.02  | 2.02  | 2.06  | 1.99  | 1.97  |

A—Mass.: 5 g. in 250 cc. of dilute HCl. R. I.: 1 g. in 10 cc. of HCl+30 cc of HNO<sub>3</sub>.

B—1 g. washed with 200 cc. of boiling water.

C—1 g. in 250 cc. of boiling water.

D—1 g. dried at 120° C., washed with 250 cc. of cold water.

E—1 g. washed with 200 cc. of hot 2% NH<sub>4</sub>Cl.

F—2 g. with 100 cc. hot 2% citric acid, 60 min.

G—2 g. with 100 cc. hot neutral ammonium citrate, 1.09 sp. gr., 60 min.

H—2 g. with 100 cc. of boiling 2% NH<sub>4</sub>Cl+15 cc. of satd. NH<sub>4</sub> oxalate solution.

I—2 g. 10 min. with 185 cc. of boiling water+15 cc. of satd. NH<sub>4</sub> oxalate solution.

J—Same as "I," but using 1 g. sample.

K—Same as "I," but using 1 g. sample and boiled 75 min.

L—Same as "I," but using 1 g. sample and heating 60 min. at 80° C.

M—Same as "I," but using 40 cc. of the NH<sub>4</sub> oxalate solution.

N—Average percentage of MgO from ingredients other than those usually considered magnesium carriers and listed in table heading.

\* Blank assumed. Complete formulas for these mixtures were not furnished.

magnesia were the carriers intended to supply magnesium. These ingredients were analyzed separately. Other ingredients carried small quantities of magnesium. Therefore a second mixture was made for each sample to contain the proportionate parts of all ingredients known to have small quantities of magnesium, and not mentioned specifically as carriers. Magnesia was determined in these mixtures just as in the complete formulas, and results were calculated on the basis of the complete formula and subtracted as a blank. This blank was slightly larger for acid-soluble magnesium than for other solvents, but most of the magnesia was soluble in water, and all of the blank results were remarkably uniform. For the purposes of this study an average of the blanks for all solvents from the two laboratories was used in studying the effects of individual solvents.

The results are compiled in Table 6. Acid-soluble magnesium oxide from seven samples agreed well with the percentages calculated from the formulas. Two of the samples, 4-12-4 (2) and 6-8-6, could be reconciled with the total magnesium oxide found by assuming more dolomite to be present in the mixture than was listed in the formula. Data were available to justify the assumption. In two cases in which the acid-soluble magnesia was less than expected, the discrepancy could not be explained. These samples, 8-16-20 and 7-16-20, were used only to show relative solubility in ammonium oxalate under various conditions.

Water-soluble magnesium oxide has been suggested as a desirable fraction, although it is not claimed to represent the availability of a mixture. In general, it is considered more rapidly available than less soluble forms, and has been guaranteed in certain brands by some manufacturers. The principal sources are calcined kieserite, which is chiefly magnesium sulfate; and sulfate of potash-magnesia, a double sulfate. Both of these materials are in demand for supplying quickly available magnesium, and are sold on a water-soluble basis. As has been shown, all of the magnesium oxide in kieserite is water-soluble. The solubility of magnesium oxide in the double sulfate depends upon conditions of extraction, especially the quantity of water and the time.

The method of extraction used was suggested by Haskins; it is based on experience in examining fertilizers sold in Massachusetts that have carried this guaranty. A 1 gram charge was weighed on an 11 cm. No. 42 Whatman filter and washed with boiling water to a volume of 250 cc., cooled, and made to volume with water. Haskins then proceeds with the determination by the sodium acetate method as follows:

To an aliquot corresponding to 0.5 gram of fertilizer add 1 cc. of 6%  $\text{FeCl}_3$  for every 2.5 of water-soluble  $\text{P}_2\text{O}_5$ . Heat almost to boiling. Make slightly alkaline with  $\text{NH}_4\text{OH}$ , using bromothymol blue as indicator. Make neutral with dilute acetic acid, add 2 grams of Na acetate, boil 2 minutes, allow to stand 10 minutes, and filter. Wash the precipitate five times with hot water. Dissolve the precipitate in



warm HCl (1+4) on filter paper, washing with hot H<sub>2</sub>O to bulk of 50 cc. Reprecipitate as before, filter, and wash thoroughly. Combine filtrates, evaporate to a bulk of about 50 cc., make slightly alkaline with NH<sub>4</sub>OH, precipitate Ca with a saturated solution of NH<sub>4</sub> oxalate, and digest on the steam bath 30 minutes. Allow to cool, filter, wash thoroughly, and dissolve the precipitate in hot HCl (1+1) on paper, washing five times with hot water. Reprecipitate the Ca as before, filter, and wash. Combine the filtrates, evaporate to a small bulk, transfer to a Pt or silica dish, evaporate to dryness on the steam bath, and finish in a hot air bath so as to avoid serious spattering when burning off the ammonia compounds. Drive off the ammonia salts at gentle heat until danger of spattering has ceased, then at a full red heat. Cool, and add 5 cc. of HCl (1+1) and 25 cc. of hot water. Unless solution is complete, filter, and wash into a 150 cc. beaker, make slightly alkaline with NH<sub>4</sub>OH, and precipitate the magnesium with a solution of sodium phosphate, observing the same precautions as in the determination of P<sub>2</sub>O<sub>5</sub>.

At the Rhode Island station the magnesium in the water extract was determined by the Hoffman method, that is evaporating the entire extract to dryness and adding 5 cc. of HNO<sub>3</sub>, followed by H<sub>2</sub>SO<sub>4</sub> as in the procedure recommended in this report for total magnesium oxide. Manganese should be determined in the final precipitate if present. Either procedure should be satisfactory for analysis of the extract, but it is suggested that uniformity would be gained if all laboratories adopt for mixed fertilizers the extraction method that Haskins recommends. Such carriers as kieserite and sulfate of potash-magnesia should be placed in water and boiled. The first is easily soluble, but Haskins obtained only about 75 per cent of the magnesium oxide in sulfate of potash-magnesia by the water extraction that he used. This product needs special study.

TABLE 7.—Percentage recovery of water-soluble MgO

| FERTILIZER<br>GRADE | MgO CALCULATED FROM FORMULA |          |           |                                   | RECOVERY* OF MgO (PER CENT OF THAT ADDED<br>IN KIESERITE AND SULFATE OF POTASH-<br>MAGNESIA) |                      |  |
|---------------------|-----------------------------|----------|-----------|-----------------------------------|--|----------------------|--|
|                     | MAGNESIAN<br>LIMESTONE      | CALNITRO | KIESERITE | SULFATE<br>OF POTASH-<br>MAGNESIA | FROM ALL<br>INGREDIENTS  | FROM Mg.<br>CARRIERS | FROM Mg. CARRIERS<br>ASSUMING 75%<br>SOLUBILITY OF MgO<br>IN SULFATE OF<br>POTASH-MAGNESIA |
|                     | per cent                    | per cent | per cent  | per cent                          |  |                      |  |
| 6-8-6               | 2.20                        | 1.21     |           |                                   |  |                      |  |
| 8-16-16             |                             | 1.23     | 1.58      |                                   | 100  | 93                   |  |
| 8-12-20             |                             | 1.18     |           | 1.82                              | 71   | 65                   | 94   |
| 3-8-5               | 1.02                        |          |           | 1.01                              | 155†   | 142†                 | 208†   |
| 8-16-14             | 1.39                        | 0.62     | 1.32      |                                   | 117  | 105                  |  |
| 4-8-10              |                             | 0.17     | 0.80      | 1.41                              | 91   | 88                   | 105  |

\* Rhode Island results. These are higher than those reported by Haskins and higher than those obtained in Rhode Island after samples had been stored for several weeks.

† Portion of dolomite apparently decomposed by NH<sub>3</sub>-NH<sub>4</sub>OH liquor during curing.

In Table 7 the per cent apparent recovery of water-soluble magnesium oxide is shown. The analyses used were higher than those obtained by

Haskins and higher than those found at the Rhode Island station after storage of the samples. The recoveries are erratic. Using the magnesium contributed by all ingredients, guarantees based on the formula would have been satisfactory in all cases but one. Subtracting the blank, the water-soluble magnesium oxide supposed to have been added was not recovered in 3 of 7 cases. Kieserite gave better results than did sulfate of potash-magnesia. If only 75 per cent of magnesium oxide in the latter is considered water-soluble under the conditions of this extraction, the recovery is more satisfactory. Had the lower analyses obtained later in the season been used, the recoveries would have been much lower. The apparent decrease in solubility because of reactions in the mixture is shown in Table 8. The experience of both Haskins and the writer lead to the be-

TABLE 8.—*Changes in water-soluble MgO in fertilizers during storage*  
(Results expressed in percentage)

| GRADE   | JULY 20 | AUGUST 4 | AUGUST 10 | AUGUST 17 | NOVEMBER 1 |
|---------|---------|----------|-----------|-----------|------------|
| 8-16-16 | 1.60    | 1.35     |           |           | 1.23       |
| 8-12-20 | 1.88    | 1.38     | 1.28      | 1.12      | 1.44       |
| 8-16-20 | 1.60    | 1.43     | 1.35      | 1.51      | 1.33       |
| 7-16-20 | 1.70    | 1.60     | 1.46      |           | 1.29       |

lief that no official method for water-soluble magnesium oxide should be adopted now. The practice of determining water-soluble magnesium oxide in such fertilizer ingredients as kieserite and sulfate of potash-magnesia, sold at a premium because of a general belief that this magnesium oxide is rapidly available, should be continued until more plant and soil research establishes the true relationships.

The ammonia-ammonium hydroxide solution used in the base mixtures of samples 4-12-4 (1) and 3-8-5 changed considerable amounts of magnesium from dolomite into a water-soluble compound.

#### RECOVERY OF MgO FROM FERTILIZERS CONTAINING MAGNESIAN LIMESTONE

Dolomitic limestone is probably the most economical source of magnesium oxide, and it may ultimately supply most of the magnesium used in fertilizers. Its use in neutral fertilizers will greatly decrease the number of magnesium-deficient areas, although not used for that specific purpose. The rate of decomposition in the soil has been measured by MacIntire<sup>1,2</sup> and White,<sup>3</sup> after periods of a year or longer. Morgan and Salter<sup>4</sup> and Taylor and Pierre<sup>5</sup> have studied the action during shorter periods. Dolomite seems to decompose more slowly than calcic limestone, and the rate cer-

<sup>1</sup> *Soil Sci.*, 20, 403-416 (1925).

<sup>2</sup> *J. Am. Soc. Agron.*, 25, 285-297 (1933).

<sup>3</sup> *Penn. Agr. Exp. Sta. Bull.* 149 (1917).

<sup>4</sup> *Soil Sci.*, 15, 293-305 (1923).

<sup>5</sup> *J. Am. Soc. Agron.*, 27, 764-773 (1935).

tainly depends on fineness. Taylor and Pierre found no marked difference in the rate of decomposition of the four different dolomites studied. From the increase in the water-soluble magnesium of the soil in relation to that from magnesium sulfate, it appears that from 50 to 75 per cent of the magnesium in four dolomites finer than 40-mesh decomposed in a fallow soil in 85 days, or approximately one cropping period. The application was made with large quantities of acid-forming fertilizer such as might occur in the fertilizer zone.

In Table 6 are the results from extraction by a number of solvents designed to attack the dolomite in the different mixtures. Stress has been laid on ammonium oxalate solutions because of the advantage of precipitation of the calcium simultaneously with the extraction. From laboratory tests, the solubility of magnesium oxalate, even after several days in a supersaturated solution, should be sufficient to account for 7 per cent magnesium oxide from a 1 gram sample in 100 cc. of solution.

The percentage recovery of magnesium oxide from dolomite under varying conditions is reported in Table 9. The conditions specified under

TABLE 9.—*Recovery of MgO from magnesian limestone*

| FERTILIZER<br>GRADE | MgO CALCULATED FROM FORMULA      |               |                |                                     | PERCENTAGE RECOVERY OF MgO FROM MAGNESIAN LIMESTONE,<br>ASSUMING COMPLETE SOLUBILITY OF MgO IN KIESERITE<br>AND SULFATE OF POTASH-MAGNESIA |    |     |    |    |    |    |    |    |
|---------------------|----------------------------------|---------------|----------------|-------------------------------------|--|----|-----|----|----|----|----|----|----|
|                     | MAGNE-<br>SIAN<br>LIME-<br>STONE | CAL-<br>NITRO | KIESER-<br>ITE | SULFATE<br>OF<br>POTASH<br>MAGNESIA | A  | B* | F*  | G* | H* | I* | K* | L* | M* |
|                     | per cent                         | per cent      | per cent       | per cent                            |  |    |     |    |    |    |    |    |    |
| 12-16-12            | 1.61                             |               |                |                                     | 104  | 1  | 63  | 29 | 23 | 17 | 52 | 25 | 40 |
| 4-12-4 (1)          | 3.11                             |               |                |                                     | 95   | 38 | 97  | 77 | 34 | 36 | 57 | 47 | 54 |
| 4-12-4 (2)          | 1.54                             | 0.85          |                |                                     | 105  | 18 | 70  | 34 | 27 | 18 | 46 | 27 | 40 |
| 6-8-6               | 2.20                             | 1.21          |                |                                     | 104  | 6  | 72  | 38 | 24 | 14 | 35 | 26 | 43 |
| 8-16-16             |                                  | 1.23          | 1.58           |                                     | 94   | 0  | 68  | 45 | 45 | 1  | 44 | 28 | 44 |
| 8-12-20             |                                  | 1.18          |                | 1.82                                | 92   | 0  | 106 | 71 | 36 | 20 | 69 | 48 | 49 |
| 3-8-5               | 1.02                             |               |                | 1.01                                | 96   | 41 | 97  | 53 | 36 | 36 | 59 | 47 | 60 |

\* Solvents are described in Table 6. Water-soluble MgO calculated from Rhode Island results. These are higher than those reported by Haskins, and those obtained at Rhode Island after longer storage. MgO from ingredients not added as magnesium carriers subtracted in all cases.

"I" were recommended by Bartlett. This concentration probably does not dissolve sufficient dolomite, but the quantity can easily be increased by changing the conditions (Columns K, L, M). The percentage recoveries by using citric acid and neutral ammonium citrate, and by washing with ammonium chloride solution decrease in the order mentioned, but these relationships could be changed by varying the conditions. Citric acid has shown the maximum recovery and is mentioned favorably by Haskins as a solvent for more careful study. Possibly results with this solvent

might be easier to duplicate than those from the decomposition by an ammonium salt, for it may be difficult to retain the ammonium end-products to the same degree in different laboratories.

The effect of particle size on solubility in ammonium oxalate solution is recorded in Table 10. The separations were made by wet sieving. Total magnesium oxide and the magnesium oxide soluble in ammonium oxalate under the conditions described in the table were determined. The recovery reflects the fineness of the separates and may prove roughly proportional to recovery from the soil by plants.

TABLE 10.—*Solubility of different dolomite separates in ammonium oxalate solution,\* expressed as percentage of the total quantity present*

| MESH     | 10-20 | 20-40 | 40-60 | 60-100 | 100 AND FINER |
|----------|-------|-------|-------|--------|---------------|
| Sample 1 | —     | 7.6   | 10.6  | 12.5   | 45.7          |
| Sample 2 | 4.7   | 6.5   | 9.7   | 13.6   | 26.6          |
| Sample 3 | —     | 22.5† | 14.6  | 15.5   | 54.1          |

\* 0.1 g. charge, 15 cc. of saturated ammonium oxalate, 185 cc. of water, boiled for 10 min

† Aggregates of fine particles shown by microscopic examination. These did not disintegrate during the wet sieving.

### CONCLUSIONS

The Hoffman method, with slight modification used at two laboratories, has proved satisfactory for the determination of total magnesium oxide in fertilizer mixtures.

No method can be recommended for available magnesium until more results are available from plant and soil work. Several experiments now under way, supplementing present knowledge, should make it possible to devise a method in the near future.

The determination of the water-soluble fraction from fertilizer mixtures does not measure all the magnesium actually available, and often fails to recover all the water-soluble magnesium added. This solvent should continue to be used for such water-soluble ingredients as kieserite and sulfate of potash-magnesia.

Many data on the solubility of magnesium carriers have been compiled to aid in the selection of a solvent for measuring available magnesium when more plant data are published.

### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the Hoffman method for the determination of total magnesium oxide as described in this report be adopted as a tentative method, and that additional collaborative results be obtained next year.

(2) That the study of methods for the determination of available magnesia be continued.

<sup>1</sup>For report of Subcommittee A and action of the Association, see *This Journal*, 19, 49 (1936).

## REPORT ON POTASH

By H. R. KRAYBILL (Purdue University Agricultural Experiment Station, West Lafayette, Indiana), *Associate Referee*

Based on the results of collaborative studies of a modified method<sup>1</sup> for the determination of potash in mixed fertilizers which were reported last year<sup>2</sup> a recommended change in the official method was adopted (first action).<sup>3</sup> It was recommended that collaborative work on the modified method be carried out during the coming year.

After the collaborative studies were undertaken last year Kraybill and Thornton<sup>1</sup> found several unusual fertilizers which showed residues left in the crucible after the washing out of the potassium chloroplatinate that were large enough to result in appreciable errors with the modified method when the chloroplatinate was determined by direct weighing. R. C. Charlton called the attention of the associate referee to the fact that in some cases with the modified method there is a residue left after washing the potassium chloroplatinate precipitate from the crucible with water. He stated that the iron and aluminum phosphates are not completely precipitated with ammonia when the sample is boiled with ammonium oxalate. He found that the addition of 5 cc. of a 30 per cent calcium chloride solution following boiling with ammonium oxalate will overcome this difficulty. Kraybill and Thornton<sup>4</sup> have shown that the addition of 0.6 cc. of concentrated hydrochloric acid to the alcohol with which the precipitate is taken up in the platinum dish will correct this error.

## COLLABORATIVE STUDIES

*Plans and Directions.*—Arrangements were made for a collaborative study of the official and modified methods on six samples of commercial fertilizers as follows:

*Sample K 9.*—A 0-12-6 fertilizer made according to the following formula: 1,264 lbs. of 19% superphosphate (from Tennessee phosphate rock), 355 lbs. of potash salts (34%  $K_2O$ ), 30 lbs. of hydrated lime, and 353 lbs. of sand.

*Sample K 28.*—A 2-12-6 fertilizer made by the following formula: 1,300 lbs. of 18.5% superphosphate (from Tennessee rock), 250 lbs. of tobacco stems (1.8% nitrogen and 4.0% potash), 125 lbs. of garbage tankage (2.8% nitrogen), 80 lbs. of ammonium sulfate, 65 lbs. of "B" ammonia liquor, and 180 lbs. of muriate of potash (62.4% potash).

*Sample K 42.*—A 0-10-4 fertilizer made by the following formula: 1,025 lbs. of 19.5% superphosphate (from Tennessee rock), 324 lbs. of manure salts (26.0% potash), 250 lbs. of phosphate rock, and 401 pounds of sand ( $CaCO_3$ -free).

*Sample K 44.*—A 0-10-10 fertilizer made by the following formula: 1,050 lbs. of 19% superphosphate (from Tennessee rock), 210 lbs. of muriate of potash (60.0%

<sup>1</sup> *This Journal*, 18, 260-281 (1935).

<sup>2</sup> *Ibid.*, 237-243.

<sup>3</sup> *Ibid.*, 63.

<sup>4</sup> Unpublished.

K<sub>2</sub>O), 310 lbs. of manure salts (26.0% K<sub>2</sub>O), 20 lbs. of hydrated lime, and 410 lbs. of sand (15% CaCO<sub>3</sub>).

*Sample K 59.*—A laboratory mixture prepared by the following formula: 500 grams of mono-calcium phosphate, 500 grams of dicalcium phosphate, 500 grams of tricalcium phosphate, 1,000 grams of calcium sulfate, 100 grams of AlCl<sub>3</sub>, 100 grams of FeCl<sub>3</sub>, and 900 cc. of KCl solution containing 141.75 grams of K<sub>2</sub>O. The theoretical per cent of K<sub>2</sub>O is 5.06.

*Sample K 60.*—A laboratory mixture prepared by the following formula: 2,500 grams of superphosphate, 100 grams of AlCl<sub>3</sub>, 100 grams of FeCl<sub>3</sub>, and 900 cc. of KCl solution containing 141.75 grams of K<sub>2</sub>O. The theoretical per cent is 5.20.

The following directions were sent to each collaborator:

Determine potash in each of the following samples: K 9 (6.0%), K 28 (7.0%), K 42 (4.0%), K 44 (10.0%), K 59 (5.0%), and K 60 (5.0%) by the following methods (the approximate percentages of K<sub>2</sub>O are given in parentheses):

1. *Official Method, Methods of Analysis, A.O.A.C., 1930, 25–26, 42 (a) and 43 (a).*

2. *Modified Method*

(a) *Preparation of solution*—Place 2.5 grams of the sample in a 250 cc. volumetric flask, add 125 cc. of H<sub>2</sub>O and 50 cc. of saturated NH<sub>4</sub> oxalate solution, boil for 30 minutes, add a slight excess of NH<sub>4</sub>OH, cool, dilute to the mark, and filter.

(b) *Determination*—To a 25 or 50 cc. aliquot, add 1 cc. of potash-free normal NaOH for each 25 cc. of aliquot, 1–2 cc. of H<sub>2</sub>SO<sub>4</sub> (1+1), and 6 to 8 granules of granulated sugar; evaporate to dryness, and ignite to whiteness at low temperature. Maintain a dull red heat until the residue is perfectly white. Dissolve the residue in hot H<sub>2</sub>O, using at least 20 cc. for each decigram of K<sub>2</sub>O present, and add a few drops of HCl and then an excess of the Pt solution. Evaporate on a water bath to a thick paste, avoiding exposure to NH<sub>3</sub>. Treat the residue with approximately 6 cc. of 80% alcohol, adding 0.6 cc. of conc. HCl. Proceed from here as directed in the official method.

3. *Official Method.*—Except use 95% alcohol for taking up the K<sub>2</sub>PtCl<sub>6</sub> precipitate and for the first washing, that is before washing with the NH<sub>4</sub>Cl solution.

4. *Modified Method.*—Except use 95% alcohol containing 10 cc. of conc. HCl per 100 cc. of alcohol for taking up the K<sub>2</sub>PtCl<sub>6</sub> precipitate and for the first washing, that is before washing with the NH<sub>4</sub>Cl solution.

*Caution!* Make all ignitions carefully at low temperatures to avoid losses by spattering or volatilization.

## RESULTS

The results of the analyses made by the following twenty-three collaborators are presented in Table 1. In practically every case the results are the averages of several determinations.

### COLLABORATORS

1. D. R. Bowman, Lafayette, Ind.
2. A. N. Lineweaver, Norfolk, Va.
3. W. L. Adams, Kingston, R.I.
4. C. C. Howes, Baltimore, Md.
5. P. R. Bidez and T. H. Burton, Auburn, Ala.
6. R. M. Smith, Tallahassee, Fla.
7. H. R. Allen, Lexington, Ky.
8. Percy O'Meara, Lansing, Mich.
9. L. H. VanWormer, College Park, Md.

TABLE 1.—*Collaborative results*

| COLLABORATOR         | WASHED WITH 80% ALCOHOL      |        |       | WASHED WITH 95% ALCOHOL,<br>FIRST WASHING |        |        | EFFECT OF USING 95%<br>ALCOHOL FOR FIRST WASHING |                    |
|----------------------|------------------------------|--------|-------|---|--------|--------|--|--------------------|
|                      | OFFICIAL MODIFIED DIFFERENCE |        |       | OFFICIAL MODIFIED DIFFERENCE              |        |        | OFFICIAL<br>METHOD                               | MODIFIED<br>METHOD |
|                      | METHOD                       | METHOD |       | METHOD                                    | METHOD |        |  |                    |
| Sample K 9 (0-12-6)  |                              |        |       |   |        |        |  |                    |
| 1                    | 5.79                         | 6.22   | +0.43 | 5.75                                      | 6.24   | +0.49  | -0.04  | +0.02              |
| 2                    | 5.80                         | 6.16   | +0.36 | 5.84                                      | 6.16   | +0.32  | +0.04  | 0.00               |
| 3                    | 5.92                         | 6.35   | +0.43 | 6.04                                      | 6.18   | +0.14  | +0.12  | -0.17              |
| 4                    | 5.78                         | 6.18   | +0.40 | 5.99                                      | 6.13   | +0.14  | +0.21  | -0.05              |
| 5                    | 5.75                         | 6.37   | +0.62 | 6.16                                      | 6.43   | +0.27  | +0.41  | +0.06              |
| 6                    | 6.18                         | 6.23   | +0.05 | 6.11                                      | 6.28   | +0.17  | -0 07  | +0.05              |
| 7                    | 5.80                         | 6.10   | +0.30 | 5.75                                      | 6.30   | +0.55  | -0.05  | +0.20              |
| 8                    | 6.21                         | 6.37   | +0.16 | 6.17                                      | 6.50   | +0.33  | -0.04  | +0.13              |
| 9                    | 5.98                         | 6.19   | +0.21 | 6.05                                      | 6.02   | -0.03  | +0.07  | -0.17              |
| 10                   | 5.85                         | 6.10   | +0.25 | 5.97                                      | 6.40   | +0.43  | +0.12  | +0.30              |
| 11                   | 5.68                         | 6.07   | +0.39 | 5.73                                      | 6.11   | +0.38  | +0.05  | +0.04              |
| 12                   | 6 01                         | 6.02   | +0.01 | 5.99                                      | 6.07   | +0.08  | -0.02  | +0.05              |
| 13                   | 5.68                         | 5.86   | +0.18 | 5.99                                      | 6.09   | +0.10  | +0.31  | +0.23              |
| 15                   | 5.94                         | 6.30   | +0.36 | 5.99                                      | 6.28   | +0.29  | +0.05  | -0.02              |
| 16                   | 5.92                         | 6.21   | +0 29 | 5.94                                      | 6.30   | +0.36  | +0.02  | +0.09              |
| 17                   | 5.89                         | 6.23   | +0.34 | 5.86                                      | 5.93   | +0.07  | -0.03  | -0.30              |
| 18                   | 5.93                         | 6.20   | +0.27 | 5.97                                      | 6.23   | +0.26  | +0 04  | +0.03              |
| 19                   | 5 94                         | 6.05   | +0.11 | 5.88                                      | 6.28   | +0.40  | -0.06  | +0.23              |
| 20                   | 5.91                         | 6.20   | +0.29 | 5.95                                      | 6.20   | +0.25  | +0 04  | 0.00               |
| 21                   | 6.02                         | 6.03   | +0.01 | 6.07                                      | 6 12   | +0.05  | +0.05  | +0.09              |
| 22                   | 6.04                         | 6.10   | +0.06 | 6.07                                      | 6 07   | 0.00   | +0.03  | -0.03              |
| 23                   | 5.98                         | 6.23   | +0.25 | 5.93                                      | 6.16   | +0.23  | -0.05  | +0.07              |
| 24                   | —                            | —      | —     | 5.89                                      | 6.16   | +0.27  | —  | —                  |
| Maximum              | 6.21                         | 6.37   | +0.62 | 6.17                                      | 6.50   | +0.55  | +0.41  | +0.30              |
| Minimum              | 5.68                         | 5.86   | +0.01 | 5 73                                      | 5 93   | -0.03  | -0.07  | -0.30              |
| Average              | 5.91                         | 6.17   | +0.26 | 5.96                                      | 6.20   | +0.24  | +0.05  | +0.04              |
| Sample K 28 (2-12-6) |                              |        |       |   |        |        |  |                    |
| 1                    | 7.36                         | 7.50   | +0.14 | 7.36                                      | 7.56   | +0.20  | 0.00   | +0.06              |
| 2                    | 7.24                         | 7.60   | +0.36 | 7.44                                      | 7.66   | +0.22  | +0.20  | +0.06              |
| 3                    | 7 52                         | 7.29   | -0.23 | 7.48                                      | 7.65   | +0.17  | -0.04  | +0.36              |
| 4                    | 7.14                         | 7.51   | +0.37 | 7.43                                      | 7.43   | 0.00   | +0.29  | -0.08              |
| 5                    | 7.46                         | 7.57   | +0.11 | 7.26                                      | 7.64   | +0.38  | -0.20  | +0.07              |
| 6                    | 7.76                         | 7.76   | 0.00  | 7.71                                      | 7.61   | -0.10  | -0.05  | -0.15              |
| 7                    | 7.00                         | 7.11   | +0.11 | 7.02                                      | 7.10   | +0.08  | +0.02  | -0.01              |
| 8                    | 7.57                         | 7.63   | +0.06 | 7.41                                      | 7.61   | +0.20  | -0.16  | -0.02              |
| 9                    | 7.37                         | 7.72   | +0.35 | 7.41                                      | 7.47   | +0.06  | +0.04  | -0.25              |
| 10                   | 7.37                         | 7.40   | +0.03 | 7.26                                      | 7.26   | 0.00   | -0.11  | -0.14              |
| 11                   | 7.09                         | 7.56   | +0.47 | 7.27                                      | 7.81   | +0.54  | +0.18  | +0.25              |
| 12                   | 7.42                         | 7.36   | -0.06 | 7.41                                      | 7.49   | +0.08  | -0.01  | +0.13              |
| 13                   | 7.06                         | 7.33   | +0.27 | 6.88*                                     | 7.04*  | +0.16* | —  | —                  |
| 15                   | 7.48                         | 7.63   | +0.15 | 7.55                                      | 7.45   | -0.10  | +0.07  | -0.18              |

TABLE 1.—*Collaborative results (Continued)*

| COLLABORATOR          | WASHED WITH 80% ALCOHOL |          |            | WASHED WITH 95% ALCOHOL,<br>FIRST WASHING |          |            | EFFECT OF USING 95%<br>ALCOHOL FOR FIRST WASHING |          |
|-----------------------|-------------------------|----------|------------|---|----------|------------|--|----------|
|                       | OFFICIAL                | MODIFIED | DIFFERENCE | OFFICIAL                                  | MODIFIED | DIFFERENCE | OFFICIAL   | MODIFIED |
|                       | METHOD                  | METHOD   |            | METHOD                                    | METHOD   |            | METHOD   | METHOD   |
| 16                    | 7 36                    | 7 48     | +0.12      | 7 41                                      | 7 60     | +0 19      | +0 05  | +0.12    |
| 17                    | 7.26*                   | 7.07*    | -0.19*     | 7 25                                      | 7 20     | -0 05      | —  | -        |
| 18                    | 7.35                    | 7 41     | +0.06      | 7 41                                      | 7 45     | +0.04      | +0 06  | +0.04    |
| 19                    | 7 40                    | 7 48     | +0 08      | 7.39                                      | 7.52     | +0.13      | -0 01  | +0 04    |
| 20                    | 7.39                    | 7.54     | +0.15      | 7 44                                      | 7 51     | +0 07      | +0 05  | -0 03    |
| 21                    | 7.52                    | 7 60     | +0 08      | 7.60                                      | 7 68     | +0 08      | -0 08  | +0 08    |
| 22                    | 7.44                    | 7 54     | +0 10      | 7 48                                      | 7 59     | +0.11      | -0 04  | +0 05    |
| 23                    | 7.17                    | 7 33     | +0.16      | 7.22                                      | 7 36     | +0 14      | +0 05  | +0 03    |
| 24                    | —                       | —        | —          | 7 38                                      | 7 67     | +0 29      | —  | —        |
| Maximum               | 7.76                    | 7 76     | +0.47      | 7 71                                      | 7 81     | +0.54      | +0 29  | +0.36    |
| Minimum               | 7 00                    | 7 11     | -0.23      | 7.02                                      | 7 10     | -0.10      | -0 20  | -0 25    |
| Average               | 7 36                    | 7 49     | +0 13      | 7.39                                      | 7 51     | +0 12      | +0 02  | +0 02    |
| Sample K 42 (0-10-4)  |                         |          |            |   |          |            |  |          |
| 1                     | 3 74                    | 4.28     | +0 54      | 3.90                                      | 4 35     | +0 45      | +0 16  | +0 07    |
| 2                     | 3.83                    | 4.22     | +0 39      | 4 02                                      | 4.26     | +0 24      | +0.19  | +0 04    |
| 3                     | 3 95                    | 4.37     | +0.42      | 3 96                                      | 4 33     | +0 37      | +0 01  | -0 04    |
| 4                     | 3 86                    | 4 10     | +0 24      | 4.02                                      | 4 01     | -0 01      | +0 16  | -0 09    |
| 5                     | 3 90                    | 4.44     | +0 54      | 4 17                                      | 4 39     | +0.22      | +0 27  | -0 05    |
| 6                     | 4.12                    | 4 20     | +0 08      | 4.12                                      | 4.28     | +0.16      | 0 00   | +0 08    |
| 7                     | 3.81                    | 4 10     | +0 29      | 3 96                                      | 4.24     | +0.28      | +0 15  | +0 14    |
| 8                     | 4 10                    | 4.54     | +0.44      | 3 85                                      | 4 60     | +0 75      | -0 25  | +0 06    |
| 9                     | 4 02                    | 4.30     | +0 28      | 4.08                                      | 4.04     | -0 04      | +0 06  | -0 26    |
| 10                    | 3.86                    | 4.29     | +0 43      | 3 89                                      | 4.30     | +0.41      | +0 03  | +0 01    |
| 11                    | 3.64                    | 4.40     | +0.76      | 3 74                                      | 4.55     | +0.81      | +0 10  | +0.15    |
| 12                    | 3 84                    | 4.06     | +0.22      | 3 82                                      | 4 17     | +0.35      | -0 02  | +0.11    |
| 13                    | 3.53                    | 3 85     | +0 32      | 3 36*                                     | 3.96*    | +0 60*     | —  | —        |
| 15                    | 3.87                    | 4 27     | +0.40      | 3 90                                      | 4.21     | +0 31      | +0.03  | -0 06    |
| 16                    | 4.06                    | 4.21     | +0 15      | 4.03                                      | 4.28     | +0.25      | -0 03  | +0.07    |
| 17                    | 3.85                    | 4.21     | +0 36      | 3 79                                      | 4.44     | +0 65      | -0 06  | +0 23    |
| 18                    | 3.83                    | 4 18     | +0 35      | 3.91                                      | 4 26     | +0 35      | +0 08  | +0 08    |
| 19                    | 3.80                    | 4.10     | +0.30      | 3.77                                      | 4 23     | +0 46      | -0 03  | +0 13    |
| 20                    | 3.92                    | 4.24     | +0 32      | 3 93                                      | 4 21     | +0.28      | +0 01  | -0 03    |
| 21                    | 3.93                    | 4.07     | +0.14      | 3.98                                      | 4.13     | +0.15      | +0 05  | +0 06    |
| 22                    | 3.91                    | 4.15     | +0 24      | 3.99                                      | 4 18     | +0 19      | +0 08  | +0 03    |
| 23                    | 3.71                    | 4.06     | +0 35      | 3 89                                      | 4 10     | +0.21      | +0 18  | +0.04    |
| Maximum               | 4 12                    | 4.54     | +0.76      | 4 17                                      | 4.60     | +0 81      | +0.27  | +0 23    |
| Minimum               | 3.53                    | 3.85     | +0.08      | 3.74                                      | 4 01     | -0.04      | -0 25  | -0.26    |
| Average               | 3.87                    | 4.21     | +0.34      | 3 94                                      | 4 26     | +0.32      | +0.06  | +0.04    |
| Sample K 44 (0-10-10) |                         |          |            |   |          |            |  |          |
| 1                     | 9.79                    | 9.90     | +0.11      | 9.66                                      | 10.03    | +0.37      | -0.13  | +0.13    |
| 2                     | 9.64                    | 10.12    | +0.48      | 9.84                                      | 10.20    | +0.36      | +0.20  | +0 08    |



TABLE 1.—*Collaborative results (Continued)*

| COLLABORATOR | WASHED WITH 80% ALCOHOL |          |            | WASHED WITH 95% ALCOHOL,<br>FIRST WASHING |          |            | EFFECT OF USING 95%<br>ALCOHOL FOR FIRST WASHING |          |
|--------------|-------------------------|----------|------------|---|----------|------------|--|----------|
|              | OFFICIAL                | MODIFIED | DIFFERENCE | OFFICIAL                                  | MODIFIED | DIFFERENCE | OFFICIAL   | MODIFIED |
|              | METHOD                  | METHOD   |            | METHOD                                    | METHOD   |            | METHOD   | METHOD   |
| 3            | 9.73                    | 10.45    | +0.72      | 10.73*                                    | 10.54*   | -0.19*     | —  | —        |
| 4            | 9.64                    | 9.89     | +0.25      | 9.73                                      | 9.80     | +0.07      | +0.09  | -0.19    |
| 5            | 9.62                    | 10.24    | +0.62      | 9.76                                      | 10.14    | +0.38      | +0.14  | -0.10    |
| 6            | 9.92                    | 10.10    | +0.18      | 9.83                                      | 10.05    | +0.22      | -0.09  | -0.05    |
| 7            | 9.50                    | 9.72     | +0.22      | 9.68                                      | 9.92     | +0.24      | +0.18  | +0.20    |
| 8            | 9.97                    | 10.32    | +0.35      | 9.93                                      | 10.19    | +0.26      | -0.04  | -0.13    |
| 9            | 9.86                    | 10.16    | +0.30      | 9.99                                      | 9.94     | -0.05      | +0.13  | -0.22    |
| 10           | 9.72                    | 9.95     | +0.23      | 9.81                                      | 10.04    | +0.23      | +0.09  | +0.09    |
| 11           | 9.53                    | 9.88     | +0.35      | 9.57                                      | 9.95     | +0.38      | +0.04  | +0.07    |
| 12           | 9.71                    | 9.95     | +0.24      | 9.72                                      | 10.07    | +0.35      | +0.01  | +0.12    |
| 13           | —                       | —        | —          | —   | —        | —          | —  | —        |
| 15           | 9.70                    | 10.14    | +0.44      | 9.76                                      | 9.98     | +0.22      | +0.06  | -0.16    |
| 16           | 9.70                    | 9.80     | +0.10      | 9.70                                      | 9.97     | +0.27      | 0.00   | +0.17    |
| 17           | 9.60                    | 9.49     | -0.11      | 9.77*                                     | 9.52*    | -0.25*     | —  | —        |
| 18           | 9.80                    | 9.91     | +0.11      | 9.87                                      | 9.95     | +0.08      | +0.07  | +0.04    |
| 19           | 9.75                    | 9.93     | +0.18      | 9.73                                      | 10.00    | +0.27      | -0.02  | +0.07    |
| 20           | 9.64                    | 9.96     | +0.32      | 9.68                                      | 10.00    | +0.32      | +0.04  | +0.04    |
| 21           | 10.05                   | 10.11    | +0.06      | 10.10                                     | 10.08    | -0.02      | +0.05  | -0.03    |
| 22           | 9.76                    | 10.01    | +0.25      | 9.85                                      | 10.13    | +0.28      | +0.09  | +0.12    |
| 23           | 9.47                    | 9.95     | +0.48      | 9.79                                      | 9.99     | +0.20      | +0.32  | +0.04    |
| 24           | —                       | —        | —          | 9.88                                      | 10.19    | +0.31      | —  | —        |
| Maximum      | 10.05                   | 10.45    | +0.72      | 10.10                                     | 10.20    | +0.38      | +0.32  | +0.20    |
| Minimum      | 9.47                    | 9.49     | -0.11      | 9.57                                      | 9.80     | -0.05      | -0.13  | -0.22    |
| Average      | 9.72                    | 10.00    | +0.28      | 9.79                                      | 10.03    | +0.24      | +0.06  | +0.02    |

| Sample K 59 (0-25-5) |      |      |       |       |       |        |       |       |
|----------------------|------|------|-------|-------|-------|--------|-------|-------|
| 1                    | 4.54 | 4.98 | +0.44 | 4.61  | 5.07  | +0.46  | +0.07 | +0.09 |
| 2                    | 4.48 | 4.76 | +0.28 | 4.60  | 5.07  | +0.47  | +0.12 | +0.31 |
| 3                    | 4.72 | 5.00 | +0.28 | 4.85  | 4.97  | +0.12  | +0.13 | -0.03 |
| 4                    | 4.53 | 4.85 | +0.32 | 4.67  | 4.82  | +0.15  | +0.14 | -0.03 |
| 5                    | 4.73 | 5.08 | +0.35 | 4.89  | 5.03  | +0.14  | +0.16 | -0.05 |
| 6                    | 4.85 | 4.95 | +0.10 | 4.83  | 4.99  | +0.16  | -0.02 | +0.04 |
| 7                    | 4.33 | 4.76 | +0.43 | 4.53  | 4.82  | +0.29  | +0.20 | -0.06 |
| 8                    | 4.83 | 5.26 | +0.43 | 5.02* | 5.13* | +0.11* | —     | —     |
| 9                    | 4.74 | 5.01 | +0.27 | 4.66  | 4.72  | +0.06  | -0.08 | -0.29 |
| 10                   | 4.65 | 4.94 | +0.29 | 4.47  | 4.77  | +0.30  | -0.18 | -0.17 |
| 11                   | 4.32 | 4.77 | +0.45 | 4.55  | 5.00  | +0.45  | +0.23 | +0.23 |
| 12                   | 4.65 | 4.80 | +0.15 | 4.64  | 4.93  | +0.29  | -0.01 | +0.13 |
| 13                   | 4.50 | 4.63 | +0.13 | 4.89  | 4.77  | -0.12  | +0.39 | +0.14 |
| 15                   | 4.58 | 4.97 | +0.39 | 4.64  | 4.87  | +0.23  | +0.06 | -0.10 |
| 16                   | 4.62 | 4.84 | +0.22 | 4.73  | 5.02  | +0.29  | +0.11 | +0.18 |
| 17                   | 4.41 | 4.70 | +0.29 | 4.62  | 4.76  | +0.14  | +0.21 | +0.06 |
| 18                   | 4.57 | 4.95 | +0.38 | 4.63  | 5.02  | +0.39  | +0.06 | +0.07 |
| 19                   | 4.58 | 4.97 | +0.39 | 4.59  | 4.90  | +0.31  | +0.01 | -0.07 |

TABLE 1.—*Collaborative results (Continued)*

| COLLABORATOR | WASHED WITH 80% ALCOHOL |        |            | WASHED WITH 95% ALCOHOL,<br>FIRST WASHING |        |            | EFFECT OF USING 95%<br>ALCOHOL FOR FIRST WASHING |          |
|--------------|-------------------------|--------|------------|---|--------|------------|--|----------|
|              | OFFICIAL MODIFIED       |        | DIFFERENCE | OFFICIAL MODIFIED                         |        | DIFFERENCE | OFFICIAL   | MODIFIED |
|              | METHOD                  | METHOD |            | METHOD                                    | METHOD |            | METHOD   | METHOD   |
| 20           | 4.57                    | 4.97   | +0.40      | 4.65                                      | 4.90   | +0.25      | +0.08  | -0.07    |
| 21           | 4.68                    | 4.88   | +0.20      | 4.75                                      | 4.94   | +0.19      | +0.07  | +0.06    |
| 22           | 4.67                    | 4.86   | +0.19      | 4.63                                      | 4.93   | +0.30      | -0.04  | +0.07    |
| 23           | 4.39                    | 4.70   | +0.31      | 4.37*                                     | 4.60*  | +0.23*     | —  | —        |
| Maximum      | 4.85                    | 5.26   | +0.45      | 4.89                                      | 5.07   | +0.47      | +0.39  | +0.31    |
| Minimum      | 4.32                    | 4.63   | +0.10      | 4.47                                      | 4.72   | -0.12      | -0.18  | -0.29    |
| Average      | 4.59                    | 4.89   | +0.30      | 4.67                                      | 4.92   | +0.25      | +0.09  | +0.03    |

| Sample K 60 (0-18-5) |       |       |        |       |       |        |       |       |
|----------------------|-------|-------|--------|-------|-------|--------|-------|-------|
| 1                    | 4.59  | 5.12  | +0.53  | 4.60  | 5.21  | +0.61  | +0.01 | +0.09 |
| 2                    | 4.68  | 5.10  | +0.42  | 4.80  | 5.10  | +0.30  | +0.12 | 0.00  |
| 3                    | 4.56  | 5.12  | +0.56  | 5.88* | 5.45* | -0.43* | —     | —     |
| 4                    | 4.65  | 5.00  | +0.35  | 4.67  | 4.90  | +0.23  | +0.02 | -0.10 |
| 5                    | 4.89  | 5.31  | +0.42  | 4.94  | 5.22  | +0.28  | +0.05 | -0.09 |
| 6                    | 4.91  | 5.25  | +0.34  | 4.87  | 5.29  | +0.42  | -0.04 | +0.04 |
| 7                    | 4.54  | 4.98  | +0.44  | 4.58  | 5.16  | +0.58  | +0.04 | +0.18 |
| 8                    | 4.87* | 5.69* | +0.82* | 4.87  | 5.58  | +0.71  | —     | —     |
| 9                    | 4.83  | 4.93  | +0.10  | 4.83  | 4.85  | +0.02  | 0.00  | -0.08 |
| 10                   | 4.74  | 4.96  | +0.22  | 4.92  | 5.03  | +0.11  | +0.18 | +0.07 |
| 11                   | 4.45  | 5.09  | +0.64  | 4.63  | 5.17  | +0.54  | +0.18 | +0.08 |
| 12                   | 4.70  | 5.14  | +0.44  | 4.70  | 5.21  | +0.51  | 0.00  | +0.07 |
| 13                   | 4.64  | 4.73  | +0.09  | 4.56  | 4.91  | +0.35  | -0.08 | +0.18 |
| 15                   | 4.75  | 5.28  | +0.53  | 4.76  | 5.23  | +0.47  | +0.01 | -0.05 |
| 16                   | 4.71  | 4.77  | +0.06  | 4.87  | 5.00  | +0.13  | +0.16 | +0.23 |
| 17                   | 4.56  | 4.95  | +0.39  | 4.88  | 4.88  | 0.00   | +0.32 | -0.07 |
| 18                   | 4.62  | 5.10  | +0.48  | 4.69  | 5.18  | +0.49  | +0.07 | +0.08 |
| 19                   | 4.68  | 5.09  | +0.41  | 4.71  | 5.13  | +0.42  | +0.03 | +0.04 |
| 20                   | 4.66  | 5.06  | +0.40  | 4.70  | 5.03  | +0.33  | +0.04 | -0.03 |
| 21                   | —     | —     | —      | —     | —     | —      | —     | —     |
| 22                   | 4.80  | 5.13  | +0.33  | 4.83  | 5.16  | +0.33  | +0.03 | +0.03 |
| 23                   | 4.62  | 4.98  | +0.36  | 4.77  | 5.01  | +0.24  | +0.15 | +0.03 |
| Maximum              | 4.91  | 5.28  | +0.64  | 4.94  | 5.58  | +0.71  | +0.32 | +0.23 |
| Minimum              | 4.45  | 4.73  | +0.06  | 4.56  | 4.85  | 0.00   | -0.08 | -0.10 |
| Average              | 4.68  | 5.05  | +0.37  | 4.76  | 5.11  | +0.35  | +0.07 | +0.04 |

\* Omitted from summary.

10. L. J. Savana, New Orleans, La.
11. C. A. Butt and J. R. Banks, East Point, Ga.
12. W. T. Mathis, New Haven, Conn.
13. E. F. Boyce, Burlington, Vt.
15. R. O. Powell, Richmond, Va.

16. H. B. Siems and R. C. Koch, Hammond, Ind.
17. W. B. Keller, Clemson College, S.C.
18. L. E. Horat, Lafayette, Ind.
19. H. R. DeRose, Amherst, Mass.
20. N. P. Etheredge, State College, Miss.
21. T. L. Ogier and S. E. Asbury, College Station, Texas
22. E. C. Smith, Geneva, N.Y.
23. Bernardo G. Capó, Rio Piedras, Puerto Rico
24. P. McG. Shuey, Savannah, Ga.

In every case the modified method yielded results significantly higher than did the official method, the average increases ranging from 0.13 to 0.37 per cent of potassium oxide based on the weight of the original sample.

When 95 per cent alcohol was used to take up the precipitate of potassium chloroplatinate and for the first washings the results were only slightly higher than when 80 per cent alcohol was used. For the official method the average differences ranged from 0.02 to 0.09 per cent and for the modified method from 0.02 to 0.04 per cent potassium oxide based on the original weight of the sample.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That sec. 42(a), p. 25, Mixed Fertilizers, *Methods of Analysis A.O.A.C.*, 1930, be changed to read as follows: "Place 2.5 g of the sample in a 250 cc. volumetric flask and add 125 cc. of H<sub>2</sub>O and 50 cc. of saturated NH<sub>4</sub> oxalate soln. Boil for 30 min., add a slight excess of NH<sub>4</sub>OH, and after cooling dilute to 250 cc., mix, and pass through a dry filter." This is a recommendation for a change in an official method (final action).

(2) That the first five sentences of sec. 43(a), p. 26, Mixed Fertilizers, be changed to read as follows: "Evaporate nearly to dryness a 25 or 50 cc. aliquot of soln 42(a), to which has been added sufficient potash-free normal NaOH (1–2 cc.) to prevent the formation of free phosphoric acid during ignition, add 1 cc. of H<sub>2</sub>SO<sub>4</sub> (1+1) and 6 to 8 granules of granulated sugar, evaporate to dryness, and ignite to whiteness at low temperature. Maintain a dull red heat until the residue is perfectly white. Dissolve the residue in hot H<sub>2</sub>O, using at least 20 cc. for each decigram of K<sub>2</sub>O present, and add a few drops of HCl and then an excess of the Pt soln. Evaporate on a water bath to a thick paste, avoiding exposure to NH<sub>3</sub>. Treat the residue with approximately 6 cc. of 80% alcohol, adding 0.6 cc. of HCl." This is a recommendation for a change in an official method (final action).

(3) That there be added to sec. 42(b), p. 25, the following: "When substances that interfere, such as ammonia, lime, aluminum etc., are present, proceed as directed in 42(a);" to sec. 43(b), p. 26, the following:

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 49 (1936).

If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of this soln, proceed as directed in (a)," and to sec. 43(a), p. 26, the following: "If  $\text{NH}_4\text{OH}$  and  $\text{NH}_4$  oxalate are used in preparation of the soln, proceed as directed in (a) but use 25 cc. portions of  $\text{NH}_4\text{Cl}$  soln." This is a recommendation for a change in an official method (final action).

(4) That further study be given to the following suggested change in the last two sentences of sec. 43(a), p. 26: "Weigh and remove the chloroplatinate precipitate by washing with hot water, using slight suction. Wash with 80% alcohol three times, dry as before, and weigh (loss equals  $\text{K}_2\text{PtCl}_6$ ). Calculate to  $\text{K}_2\text{O}$ ."

(5) That studies be made of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the potash determination.

The paper, entitled "Factors Affecting the Solubility of Calcined Phosphate in Ammonium Citrate and Citric Acid Solutions," presented by Jacob and Rader, will be published later.

## REPORT OF SUBCOMMITTEE ON ACIDITY AND BASICITY OF FERTILIZERS

By L. G. WILLIS (Agricultural Experiment Station,  
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The work on which this report is based was started early in 1935 with the specific objectives of determining the relative neutralizing value of finely ground rock phosphate and dolomitic limestone, and the value of dolomitic limestone of different particle sizes. Other details concerning the properties of acid and neutral fertilizers can be illustrated by the results and these will be included in the discussion.

The following assumptions are made as a basis for the discussion:

(1) The trend toward the greater use of acid-forming materials in commercial fertilizers has created a need for a quantitative means of expressing the acid-forming properties of fertilizers.

(2) The control method of analysis must be adapted especially to the commonly used fertilizer materials without introducing serious errors in the evaluation of other materials that are used to a lesser extent.

(3) The problem of fertilizer acidity is of greatest concern under conditions of heavy fertilizer usage, on soils of low exchange capacity and those where the  $\text{pH}$  is marginal to profitable agriculture. A substantial part of all the fertilizer sold in this country is used under these conditions.

It will be shown that many factors contribute to the acidity values of fertilizer materials and to the reactivity of neutralizing agents. These do

not present any insurmountable difficulties in the way of adopting specific values for purposes of control analysis.

Pierre has assigned an acidity value equivalent to one-half of the total nitrogen content of the fertilizer. This is based on an assumption that ammoniacal and organic nitrogen are converted to nitrates with moderate rapidity in the soil and used by plants largely in this form. Such data as are available from field plat experiments support Pierre's acidity values for sulfate of ammonia with only minor deviations from the general average. The calculated values for ammonium phosphate and the organic materials are somewhat more erratic but are not consistently enough higher or lower to justify the adoption of any other factor. The discrepancies are further minimized by the fact that these materials constitute a very minor part of the general run of mixed fertilizers.

One possible cause of variability in acidity values for nitrogen is suggested by some experiments on nitrification. The data were obtained from a series of soil-fertilizer mixtures incubated at constant moisture content. The fertilizer consisted of an ammonium phosphate-sulfate of ammonia-muriate of potash mixture with supplements of rock phosphates, calcium sulfate, dolomitic limestone and calcium carbonate.

A distinct increase in acidity is developed when any fertilizer containing inorganic salts is mixed with the soil. This effect is due to equilibrium conditions that are dependent on the maintenance of a high salt concentration in the soil. It is entirely eliminated when the soils are leached and is therefore not to be considered in relation to the permanent acidifying effects of fertilizers. Until this leaching takes place, however, the temporary acidity does modify the soil reaction in the zone of application of the fertilizer.

The soil used in this work had an initial  $pH$  of 5.2. With the fertilizer this  $pH$  dropped to about 4.7. At this point and up to about 5.2 no appreciable nitrification of the ammonium compounds took place within 59 days but addition of various neutralizing materials, which raised the  $pH$  above 5.5., created conditions favorable to nitrification. On strongly acid soils, therefore, it would appear that the acidity equivalent for nitrogen except that added as nitrate is distinctly less than that proposed by Pierre.

This point raises a rather fine distinction in the interpretation of equivalent acidity values for fertilizers used in strongly acid soils. If no neutralizing agent is used in potentially acid fertilizers the acidifying effect will be less than that calculated. If, on the other hand, an amount of dolomitic limestone or other alkaline agent is used to produce a potentially neutral fertilizer, nitrification will become active and the equivalent acidity of the original fertilizer will approach more nearly the calculated value. From the agronomic viewpoint this enhances the values of non-acid forming fertilizers on extremely acid soils and introduces no ob-

jectional features due to the fact that the acid values for nitrogen are low under such soil conditions. These results also show that the solvent effects of nitrification have been greatly overemphasized. Apparently the solubility of alkaline agents govern nitrification rather than the reverse.

Data from field soils fairly consistently support the conclusion that the mono basic phosphates and potash salts have no appreciable effect on soil acidity. One publication indicates that sulfate of potash increases the pH of the soil. This is of no great concern except with fertilizers for tobacco and there is no evidence that exception is of sufficient importance to merit particular attention.

As might be expected the efficiency of dolomitic limestone is definitely determined by the size of particles. Material finer than 100 mesh is distinctly superior to all coarser separates. It appears, however, that the run-of-mill dolomite finer than 10 mesh is approximately equivalent to the 60-100 mesh grade and it can be assumed that the run-of-mill finer than 20 mesh would give satisfactory results.

If possible, therefore, it will be desirable to incorporate into the analytical method some means of eliminating from the determination all liming materials coarser than 20 mesh. It will be unfortunate, however, if this limitation encourages the use of materials from which the normal proportions of the finer separates have been removed.

The value of rock phosphate as a neutralizing agent in fertilizers is apparently negligible. In a soil-fertilizer mixture, rock phosphate had no appreciable neutralizing effect even at extreme acidity. The results obtained in this work offer a satisfactory explanation for the discrepancy between the apparent availability of the phosphoric acid of phosphate rock and failure of this material to serve as a neutralizing agent. Where the fertilizer used contained ammonium phosphate the rock phosphate had no neutralizing effect and the reactivity of dolomite was definitely less than that of a fertilizer containing only ammonium sulfate and muriate of potash. With the latter mixture the phosphate rock was slightly reactive. This suggests an interpretation that available phosphates from other sources depress the solubility of the rock phosphate. From this viewpoint the apparent inconsistency between the availability of phosphoric acid in rock phosphate and the inertness of this material in mixed fertilizer is easily explained.

Whether or not this point is well taken, it offers the only picture of conditions under which rock phosphate can be effective as a neutralizing agent. If the rock phosphate is to be useful in this respect, it would be essential that soil be virtually depleted of available phosphoric acid for considerable part of each year. This condition cannot be assured, and it must therefore be concluded that rock phosphate has no neutralizing value in mixed fertilizers.

If any control method is to be adopted, a distinction must be drawn

between precision in the analytical method and in the agronomical accuracy of the results. The latter can be only approximately correct at the best. It has been shown that acidity values for nitrogenous fertilizers may be too great in strongly acid soils. Further support for this opinion is given by the fact that full neutralization by dolomitic limestone is definitely more effective on strongly acid soils than on soils at higher pH values.

From general principles it can be assumed that differences will be noted between heavy and light and between broadcast and drill applications of fertilizers. These details cannot be considered in the analytical method. If they are found to produce any appreciable deviation from expected results, the proper method of compensating for these discrepancies is through the use of slightly over- or under-neutralized fertilizers.

Acknowledgments for cooperation in this work are due F. W. Parker, E. I. DuPont de Nemours Co.; W. H. Ross, U. S. Department of Agriculture; J. H. Taylor, Jr. and W. H. Pierre, West Virginia Agricultural Experiment Station; H. B. Siems, Swift and Co.; S. D. Conner, Purdue University Agricultural Experiment Station; J. B. Smith, F. R. Pember, and B. E. Gilbert, Rhode Island Agricultural Experiment Station; J. B. Hester and H. H. Zimmerly, Virginia Truck Experimental Station; and J. R. Piland, North Carolina Agricultural Experiment Station.

#### SUPPLEMENTAL REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES

The report of Subcommittee A, *This Journal*, 19, 46 (1936), failed to include a record of the action of the Association in tentatively adopting methods for the determination of selenium in soils and in plants; for the detection of acetones, ketones, isopropyl alcohol, and tertiary butyl alcohol in spirits; and for the deletion of the method for standard alkali solution, 17(c) p. 19 of *Methods of Analysis*, 1930. The details of the methods are not being published at this time because they will appear in the fourth edition of *Methods of Analysis* to issue July 1st.

## CONTRIBUTED PAPERS

### A QUANTITATIVE STUDY OF FLUORINE DISTILLATION\*

By DAN DAHLE and H. J. WICHMANN (U. S. Food and Drug  
Administration, Washington, D. C.)

As originally published,<sup>1</sup> the Willard and Winter method for separation of fluorine by distillation as  $\text{H}_2\text{SiF}_6$  called for 50 cc. distilling flasks, 5 cc. of 60 per cent perchloric acid, and a temperature between 120° and 150° C. It was also proposed that in the presence of organic matter sulfuric acid be substituted for perchloric acid in view of the explosive character of the anhydrous form of perchloric acid.

In applying the method under different conditions it was found necessary to vary the size of the flask, the temperature of the distillation, and the amount of acid used. Therefore a study was made of the quantitative effect of such variations on the recovery of the fluorine.

All fluorine determinations were made by the peroxidized titanium method, as modified by the authors.<sup>2</sup>

#### 1. EFFECT OF INPUT VOLUME

First the boiling point of known mixtures of acid and water was determined to establish the connection between boiling point and "input volume" (i.e., the total volume present in the flask when the temperature of distillation has been reached). The results are shown in Table 1.

TABLE 1.—*Boiling point of mixtures of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}$*

| B. P. | $\text{H}_2\text{SO}_4$ | $\text{H}_2\text{O}$ | INPUT VOLUME |
|-------|-------------------------|----------------------|--------------|
| °C.   | cc                      | cc                   | cc           |
| 125   | 10                      | 16                   | 26           |
| 135   | 10                      | 11.5                 | 21.5         |
| 145   | 10                      | 8.5                  | 18.5         |
| 155   | 10                      | 6.5                  | 16.5         |

Next, mixtures giving different input volumes but containing the same quantities of fluorine as NaF were distilled at constant temperature to a constant output volume. The conditions of the experiment were—

|                         |                     |
|-------------------------|---------------------|
| <i>Temperature:</i>     | Constant at 135° C. |
| <i>Output volume:</i>   | Constant at 25 cc.  |
| <i>Fluorine as NaF:</i> | Constant at 0.5 mg. |
| <i>Input volume:</i>    | Varying.            |

\* This paper is part of a thesis submitted by the senior author to the American University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, **5**, 7 (1933)

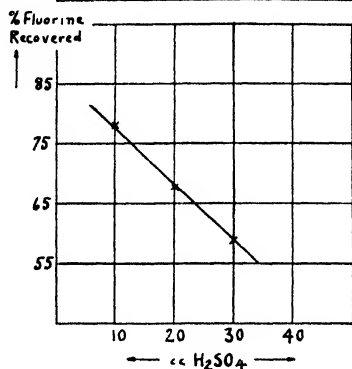
<sup>2</sup> *This Journal*, **16**, 612 (1933)



## NaF

Effect of Varying Input Volume  
on Fluorine Recovery

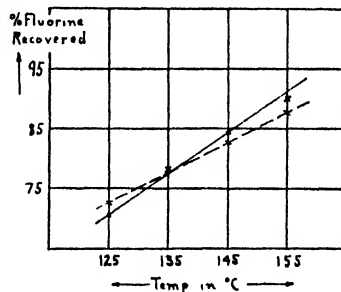
| cc H <sub>2</sub> SO <sub>4</sub> | cc Input Vol | cc Output Vol | % Recovery |
|-----------------------------------|--------------|---------------|------------|
| 10                                | 21.5         | 2.5           | 78.0       |
| 20                                | 43.0         | 2.5           | 67.8       |
| 30                                | 64.5         | 2.5           | 58.6       |



## NaF

Effect of Temperature of Distillation  
on Fluorine Recovery

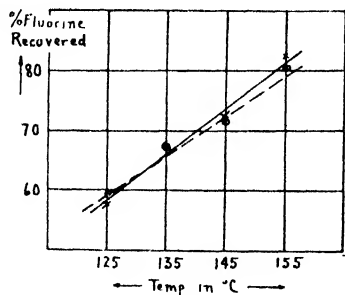
| Input Vol | Temp °C | % Recovery | % Recov Corr |
|-----------|---------|------------|--------------|
| 26.0      | 125     | 70.5       | 72.5         |
| 21.5      | 135     | 78.0       | 78.0         |
| 18.5      | 145     | 84.0       | 82.6         |
| 16.5      | 155     | 89.8       | 87.5         |



## NaF

Effect of Temperature of Distillation  
on Fluorine Recovery

| Input Vol | Temp °C | % Recovery | % Recov corr |
|-----------|---------|------------|--------------|
| 52 cc     | 125     | 57.4       | 59.4         |
| 43 "      | 135     | 67.8       | 67.8         |
| 37 "      | 145     | 72.8       | 71.4         |
| 33 "      | 155     | 82.4       | 80.4         |



## NaF

Effect of Varying Output Volume  
on Fluorine Recovery

| Temp °C | Output Vol, cc | % Recovery Found | % Recovery Calc |
|---------|----------------|------------------|-----------------|
| 125     | 10             | 40.4             | 38.3            |
| 125     | 25             | 69.4             | 70.0            |
| 125     | 50             | 90.8             | 91.0            |
| 125     | 100            | 99.0             | 99.8            |

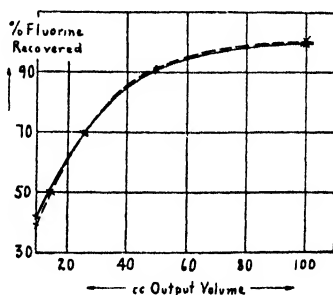


CHART 1

CHART 3

CHART 2

CHART 4

The results are shown in Table 2 and Chart 1.

TABLE 2.—*Effect of input volume on recovery*

| H <sub>2</sub> SO <sub>4</sub> | INPUT VOLUME | RECOVERY (AV.) |
|--------------------------------|--------------|----------------|
| cc.                            | cc.          | per cent       |
| 10                             | 21.5         | 78.0           |
| 20                             | 43.0         | 67.8           |
| 30                             | 64.5         | 58.6           |

From Chart 1 it was concluded that the recovery stood in inverse proportion to the input volume, *i.e.*, the amount of acid used. Quantitatively it appeared that the recovery in 25 cc. output volume decreased 1 per cent for each increase in input volume, corresponding to 1 cc. of sulfuric acid.

## 2. VARIATION WITH TEMPERATURE OF DISTILLATION

Willard and Winter reported complete recoveries in a 50-75 cc. distillate. Other investigators, among them Reynolds,<sup>1</sup> Wichmann and Dahle,<sup>2</sup> Bonnar,<sup>3</sup> and Maynard,<sup>3</sup> failed to confirm this finding and recommended larger output volumes. As the writers considered that the wide variation in temperature, 120°-150° C., might be a contributory cause of these discrepancies, they made experiments under the following conditions:

*Output volume:* Constant at 25 cc.  
*Fluorine as NaF:* Constant at 0.5 mg.  
*Input volume:* Varying with temperature.  
*Temperature:* Varying.

The results are shown in Table 3 and in Charts 2 and 3.

TABLE 3.—*Effect of temperature of distillation*

| TEMPERATURE | H <sub>2</sub> SO <sub>4</sub> | INPUT VOLUME | RECOVERY |   |
|-------------|--------------------------------|--------------|----------|---|
|             |                                |              | DIRECT   | CORRECTED FOR CHANGE<br>IN INPUT VOLUME |
| °C.         | cc.                            | cc.          | per cent | per cent                                |
| 125         | 10                             | 26           | 70.5     | 72.5                                    |
| 135         | 10                             | 21.5         | 78.0     | 78.0                                    |
| 145         | 10                             | 18.5         | 84.0     | 82.6                                    |
| 155         | 10                             | 16.5         | 89.8     | 87.5                                    |
| 125         | 20                             | 52           | 57.4     | 59.4                                    |
| 135         | 20                             | 43           | 67.8     | 67.8                                    |
| 145         | 20                             | 37           | 72.8     | 71.4                                    |
| 155         | 20                             | 33           | 82.4     | 80.1                                    |

<sup>1</sup> *This Journal*, 17, 323 (1934).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Private communication.

The full lines of the charts indicate the total variation in recovery with temperature. This variation is the sum of two factors, working in the same direction, the decreasing input volume (26, 21.5, 18.5, and 16.5 cc. in Chart 2 and 52, 43, 37, and 33 cc. in Chart 3) and the increased distillation temperature, the latter being the more important. The broken lines show the variation due to distillation temperature alone, corrections having been made for differences in input volume. From these charts it may be concluded that between 125° and 155° C. the recovery in 25 cc. output volume is increased 0.6–0.8 per cent for each rise of 1° C. in the distillation temperature.

The question may be raised: Why stop at 155° C.? Why not go still further? It was found that at 155° C. and above an increasing quantity of sulfuric acid is broken down with the formation of sulfur trioxide, the presence of which (as  $\text{H}_2\text{SO}_4$ ) in the distillate will cause a serious interference in the determination of the isolated fluorine. A distillation above 145° C., therefore, should be resorted to only in exceptional cases.

### 3. VARIATION WITH OUTPUT VOLUME

The quantitative variation of recovery with output volume was studied under the following conditions:

|                         |                     |
|-------------------------|---------------------|
| <i>Temperature:</i>     | Constant at 125° C. |
| <i>Input volume:</i>    | Constant at 26 cc.  |
| <i>Fluorine as NaF:</i> | Constant at 0.5 mg. |
| <i>Output volume:</i>   | Varying.            |

The results are shown in Table 4 and Chart 4. The full-line curve covers the recovery values actually found, whereas the broken-line curve refers to the calculated values. The striking resemblance of the full-line curve to the curve obtained by the plotting of the reaction rate of a uni-molecular reaction suggests the possibility of a mathematical treatment of the process.

TABLE 4.—*Effect of output volume on recovery*

| OUTPUT VOLUME | RECOVERY |             |
|---------------|----------|-------------|
|               | FOUND    | CALCULATED* |
| cc.           | per cent | per cent    |
| 10            | 40.4     | 38.3        |
| 25            | 69.4     | 70.0        |
| 50            | 90.8     | 91.0        |
| 100           | 99.0     | 99.2        |

\* For calculations see subsequent paragraphs.

If, in the equation for a uni-molecular reaction,

$$k = \frac{1}{t} \ln \frac{c}{c-x}, \text{ or } K = \frac{1}{t} \log \frac{c}{c-x},$$

the analyst substitutes for the time rate,  $t$ , a space rate, *i.e.*, makes  $t$  equal the number of cc. distillate collected (output volume), and allows  $c$  to represent the original concentration of fluorine and  $c-x$  the concentration remaining in the distilling flask after  $t$  cc. has been distilled, the following values for  $K$  will be obtained, corresponding to the various output volumes:

$$K_{10} = 0.02250, K_{25} = 0.02056, K_{50} = 0.02072, K_{100} = 0.02000.$$

The average of these four values is  $K = 0.020945$ .

By using this value for  $K$  and substituting the experimental values for  $t$  and  $c$  in the above equation, values of  $x$  corresponding to the various output volumes can be obtained. If, however, the distillation process can be expressed mathematically by the modified equation of the uni-molecular reaction suggested above, the recovery should vary with the amount of distillate collected, regardless of the time needed for the collection. The accuracy of this supposition was checked under the following conditions:

|                              |                     |
|------------------------------|---------------------|
| <i>Temperature:</i>          | Constant at 138° C. |
| <i>Input volume:</i>         | Constant at 42 cc.  |
| <i>Fluorine as NaF:</i>      | Constant at 0.5 mg  |
| <i>Output volume</i>         | Constant at 50 cc.  |
| <i>Time of Distillation:</i> | Varying.            |

The results are shown in Table 5.

TABLE 5.—*Effect of distilling rate on recovery*

| TIME | OUTPUT VOLUME | F ADDED | F RECOVERED |          |
|------|---------------|---------|-------------|----------|
| min. | cc            | mg.     | mg.         | per cent |
| 4    | 50            | 0.5     | 0.361       | 72.2     |
| 9    | 50            | 0.5     | 0.370       | 74.0     |
| 17   | 50            | 0.5     | 0.370       | 74.0     |
| 35   | 50            | 0.5     | 0.357       | 71.3     |

Furthermore, the percentage recovery should be independent of the original concentration of fluorine in the flask. Experimentally this was studied under the following conditions:

|                         |                     |
|-------------------------|---------------------|
| <i>Input volume:</i>    | Constant at 52 cc.  |
| <i>Output volume:</i>   | Constant at 25 cc.  |
| <i>Temperature:</i>     | Constant at 125° C. |
| <i>Fluorine as NaF:</i> | 0.5 mg. and 1.0 mg. |

The results are shown in Table 6.

TABLE 6.—*Effect of original fluorine concentration on recovery*

| FLUORINE CONCENTRATION |       | RECOVERY IN 25 CC. |  |
|------------------------|-------|--------------------|--|
| mg.                    | mg.   | per cent           |  |
| 0.5                    | 0.257 | 51.4               |  |
| 1.0                    | 0.523 | 52.3               |  |

The discrepancy, 0.9 per cent, is well within the error of the method of determination.

#### 4. SIZE OF DISTILLING FLASK

While it is recognized that this factor may be a resultant of several factors, an effort was made to give it at least an approximately quantitative expression.

Distillations were carried out under identical conditions, first in a 125 cc. Claissen flask and then in a 500 cc. flask. The results are shown in Table 7.

TABLE 7.—*Effect of varying size of the distilling flask*

| H <sub>2</sub> SO <sub>4</sub> USED | OUTPUT VOLUME | TEMPERATURE | RECOVERY FROM |               |
|-------------------------------------|---------------|-------------|---------------|---------------|
|                                     |               |             | 125 CC. FLASK | 500 CC. FLASK |
| cc.                                 | cc.           | °C.         | per cent      | per cent      |
| 50                                  | 150           | 130         | 93            | 90            |
| 50                                  | 150           | 130         | 93            | 80            |
| 50                                  | 100           | 135         | 82            | 76            |
| 50                                  | 100           | 135         | 82            | 76            |

It may be concluded (Table 7) that the substitution of a 500 cc. flask for the 125 cc. size results in a decrease of about 6.5 per cent in the recovery in 100–150 cc. output volume. This represents approximately 5 per cent decrease in the first 25 cc. distilled.

#### 5. RETARDING INFLUENCES

(a) *Gelatinous silica*.—In their original paper Willard and Winter pointed out that the presence of gelatinous silica and aluminum salts retarded the evolution of fluorine during the distillation. In the presence of 100 mg. of gelatinous silica they found a 92 per cent recovery of fluorine under conditions that in the absence of silica yielded complete recoveries. The writer's check gave a 78 per cent recovery under the following conditions:

|  |   |
|--|---|
| <i>Temperature:</i>                          | Constant at 135° C.                                       |
| <i>Input volume:</i>                         | Constant at 21.5 cc.                                      |
| <i>Output volume:</i>                        | Constant at 200 cc.                                       |
| <i>Fluorine as NaF:</i>                      | 0.5 mg.   |
| <i>Retarding influence, SiO<sub>2</sub>:</i> | 2 cc. of a 10% soln of Na <sub>2</sub> SiO <sub>3</sub> . |

(b) *Aluminum salts*.—The retarding influence of aluminum was studied under the following conditions:

|                             |  |
|-----------------------------|--|
| <i>Temperature:</i>         | Constant at 135° C.  |
| <i>Input volume:</i>        | Constant at 21.5 cc.   |
| <i>Output volume:</i>       | Constant at 200 cc.  |
| <i>Fluorine as NaF:</i>     | 0.5 mg.  |
| <i>Retarding influence:</i> | $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ , 3 grams. |

The fluorine recovery found under these conditions was 69.4 per cent. Further data on the retarding influence of aluminum salts will be found in a subsequent report.<sup>1</sup>

(c) *Ferric and ferrous salts*.—The similarity in analytical behavior between iron and aluminum salts, coupled with the fact that an iron cryolite exists, made it appear advisable to investigate the possible effect of iron salts, particularly ferric.

Experiments conducted with ferric alum,  $\text{Fe}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$ , and ferrous sulfate,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , failed, however, to show any retarding influences under conditions where equivalent amounts of aluminum would show 50 per cent retardation.

## 6. APPLICATIONS

Various practical applications may be made of this study. A rapid, semi-quantitative test for fluorine can be made by distilling under known conditions of input volume and temperature and determining the fluorine in the first 10 cc. collected. According to Chart 4 the amount found in these 10 cc. constitutes a definite percentage of the total amount present (40 per cent, if distillation is made at 125° C. with 10 cc. of sulfuric acid and a total input volume of 26 cc.). This allows an approximate estimation of the total without the necessity of making a complete distillation.

Another application deals with increasing the precision of the determination of very small quantities of fluorine. In work on foods heretofore the method of fluorine isolation has included collection of a 200 cc. distillate, 10–50 cc. of which, depending on the amount of fluorine expected, has been used for the subsequent colorimetric determination. Thus a maximum of one-fourth of the total distillate has been used for the actual determination. With very small quantities, the smallest amount possible of detection would be one-fourth of the present quantity, if all the fluorine could be recovered in a 50 cc. instead of a 200 cc. distillate. For example—

Table 2 shows that with 10 cc. of sulfuric acid and a total input volume of 21.5 cc., 78 per cent of fluorine is recovered in a 25 cc. distillate at 135° C. and that recovery increases approximately 1 per cent for each decrease in the acid of 1 cc. Hence 78+5, or 83 per cent, may be expected in 25 cc. when 5 cc. of sulfuric acid and an input volume of 10.5–11 cc. are used.

<sup>1</sup> *This Journal*, 19, 320 (1936).

Table 3 shows that if the distillation temperature is increased from 135° to 145° C. the recovery increases about 6 per cent. Hence 83+6, or 89 per cent, may be expected in 25 cc. at 145° C.

If these values are substituted in the equation growing out of Table 4, the expected recovery in 50 cc. may be calculated as follows:

$$k = \frac{1}{25} \log \frac{100}{100-89} = \frac{1}{50} \log \frac{100}{100-x}$$

This equation gives  $x=98.79$ , *i.e.*, 98.8 per cent recovery may be expected in a 50 cc. distillate collected at 145° C. when 5 cc. of sulfuric acid is used in the flask.

Actual experiments gave 97.0 and 100.3 per cent, respectively.

#### SUMMARY

A study was made of the various factors influencing the recovery of fluorine by the Willard and Winter distillation process.

Quantitative expressions are given to the effects on recovery of such factors as (1) the amount of non-volatile acid used in the distilling flask, (2) the temperature at which the distillation is carried out, (3) the amount of distillate collected, (4) the size of flask used, and (5) the presence of some retarding influences such as aluminum salts and gelatinous silica.

These data were applied to the development of a rapid, semi-quantitative test and to the problem of increasing the sensitivity of the fluorine determination for very small quantities.

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### THE DETERMINATION OF FLUORINE IN THE PRESENCE OF A LARGE EXCESS OF ALUMINUM IONS\*

By DAN DAHLE and H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.)

In a previous publication, the same investigators<sup>1</sup> reported a study of various factors influencing the recovery of fluorine by distillation. The retarding influence of aluminum salts mentioned by Willard and Winter<sup>2</sup> was confirmed. In order to study in greater detail the problem of separating small quantities of fluorine from large amounts of aluminum ions a series of experiments was performed along the same lines followed previously in the study of the distillation.<sup>1</sup>

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\* This paper is part of a thesis submitted by the senior author to the American University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>1</sup> *This Journal*, 19, 313 (1936).

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).

## 1. VARIATION IN RECOVERY WITH AMOUNT OF ALUMINUM

This was studied under the following conditions:

|  |                      |
|--|----------------------|
| <i>Temperature:</i>                                  | Constant at 135° C.  |
| <i>Input volume:</i>                                 | Constant at 21.5 cc. |
| <i>Output volume:</i>                                | Constant at 200 cc.  |
| <i>Fluorine as NaF.</i>                              | Constant at 0.5 mg.  |
| <i>Al as <math>Al_2(SO_4)_3 \cdot 18H_2O</math>.</i> | Varying.             |

The results are shown in Table 1 and Chart 1.

TABLE 1.—*Effect of varying concentrations of Al*

| $Al_2(SO_4)_3 \cdot 18H_2O$ | RECOVERY |
|-----------------------------|----------|
| grams                       | per cent |
| 1                           | 100.2    |
| 2                           | 96.0     |
| 3                           | 69.4     |
| 5                           | 48.0     |
| 7                           | 36.7     |
| 9                           | 26.6     |
| 10                          | 19.0     |

It is apparent (Table 1) that with increasing concentrations of aluminum sulfate the recovery of fluorine decreases according to a logarithmic function, *i.e.*, the logarithm of the recovery is inversely proportional to the amount of  $Al_2(SO_4)_3 \cdot 18H_2O$  present.

Comparisons of recoveries, where a certain amount of aluminum ion (0.8 gram) was added as aluminum sulfate in one case and as sodium aluminum sulfate in another, conditions of distillation and determination being otherwise equal, resulted as follows (Table 2):

TABLE 2.—*Effect of different Al salts*

| Al SALT                                    | RECOVERY |
|--|----------|
|  | per cent |
| $Al_2(SO_4)_3 \cdot 18H_2O$                | 86.2     |
| $Al_2(SO_4)_3 \cdot Na_2SO_4 \cdot 24H_2O$ | 86.5     |

The retarding influence, therefore, is apparently due to the aluminum ion itself; hence, the relation between the fluorine recovery and the amount of aluminum present may be expressed as:

$$\log \frac{C}{C_0} = k (Al),$$

where  $C$  is the amount of fluorine recovered,  $C_0$  the amount originally present,  $k$  a constant and  $(Al)$  is the concentration of aluminum ion.



## 2. SULFURIC ACID VERSUS PERCHLORIC ACID

The experiments of Willard and Winter<sup>1</sup> indicate that in the presence of 0.2 gram of aluminum (1000 mg.  $\text{AlCl}_3$ ) a collection of 250 cc. of distillate results in a fluorine recovery of 54 per cent. Chart 1 shows that 0.2 gram of aluminum (2.5 grams  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ ) corresponds to a recovery of 79 per cent in 200 cc. While each of various differences in the conditions under which the two results were obtained may contribute to the discrepancy, a question arises as to a possible difference in efficiency of sulfuric acid and perchloric acid during the distillation. To check this point, 5 grams of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  and 0.5 mg. of fluorine added as NaF were distilled under the same conditions of temperature ( $135^\circ \text{C.}$ ), input and output volumes (21.5 and 50 cc., respectively) with 10 cc. of 95 per cent sulfuric acid and 19 cc. of 60 per cent perchloric acid (molecular equivalents). The recoveries were, with sulfuric acid, 15.1 per cent; with perchloric acid 4.2 per cent.

In the separation of fluorine from aluminum salts sulfuric acid is thus shown to be the more efficient. This incidentally is not a unique situation, since in the case of thorium fluoride, practically no fluorine can be distilled off with perchloric acid at  $135^\circ \text{C.}$  (in the presence of excess thorium salt) except by a very prolonged distillation.

## 3. EFFECT OF INPUT VOLUME

The conditions of this experiment follow:

|   |  |
|---|--|
| <i>Temperature:</i>                             | Constant at $145^\circ \text{C.}$  |
| <i>Output volume:</i>                           | Constant at 100 cc.  |
| <i>Fluorine as NaF:</i>                         | Constant at 0.5 mg.  |
| <i>Concentration of <math>\text{Al}</math>:</i> | Constant at 5 g. $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ . |
| <i>Input volume:</i>                            | Varying.   |

The results are shown in Table 3 and in Chart 2.

TABLE 3.—*Effect of varying input volume*

| $\text{H}_2\text{SO}_4$ | INPUT VOLUME | RECOVERY |
|-------------------------|--------------|----------|
| cc.                     | cc.          | per cent |
| 10                      | 20.5         | 30.6     |
| 20                      | 41.0         | 20.3     |
| 30                      | 61.5         | 12.9     |

The decrease in recovery of fluorine is in direct proportion to the increase in input volume, and the recovery in a 100 cc. distillate decreases roughly 1 per cent for each increase in input volume corresponding to 1 cc. of sulfuric acid.

<sup>1</sup> *Loc. cit.*

## 4. EFFECT OF DISTILLATION TEMPERATURE

The experimental conditions follow:

|                             |  |
|-----------------------------|--|
| <i>Temperature:</i>         | Varying.   |
| <i>Input volume:</i>        | Varying with temperature.  |
| <i>Output volume:</i>       | Constant at 200° C.  |
| <i>Fluorine as NaF:</i>     | Constant at 0.5 mg.  |
| <i>Concentration of Al:</i> | Constant at 10 g. $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ . |

The results are shown in Table 4 and Chart 3. The chart shows that the recovery in 200 cc. of distillate increases about 2 per cent for each rise of 1° C. in the distillation temperature.

TABLE 4.—*Effect of distillation temperature*

| TEMPERATURE | $\text{H}_2\text{SO}_4$ | INPUT VOLUME | RECOVERY (AVERAGE) |                            |
|-------------|-------------------------|--------------|--------------------|----------------------------|
|             |                         |              | FOUND              | CORRECTED FOR INPUT VOLUME |
| °C.         | cc.                     | cc.          | per cent           | per cent                   |
| 125         | 10                      | 27.0         | 20.6               | 23.3                       |
| 132         | 10                      | —            | 32.4               | —                          |
| 135         | 10                      | 23.0         | 36.9               | 38.0                       |
| 142         | 10                      | —            | 56.7               | —                          |
| 145         | 10                      | 20.5         | 61.8               | 61.8                       |
| 152         | 10                      | —            | 81.0               | —                          |
| 155         | 10                      | 18.5         | 86.6               | 85.7                       |
| 165         | 10                      | —            | 100.               | —                          |

Of interest in this connection is the effect of sulfates. In the previous publication<sup>1</sup> it was mentioned that at 155° C. sulfuric acid breaks down with the formation of sulfur trioxide, and as a result will in part distil over with the fluorine. When aluminum sulfate is present, the amount of sulfuric acid in the distillate is greatly increased. One reason for this is the precipitation of solid (and probably anhydrous) aluminum sulfate in the distilling flask. This in turn acts as a "boiler scale" and results in local overheating, causing increased volatilization of sulfur trioxide and incidentally excessive breakage of distilling flasks.

The effect of sulfates in the colorimetric determination of fluorine by the peroxidized titanium method is to increase the color,<sup>2</sup> and thus counteract the bleaching effect exerted by the fluorine. This in turn produces low results. To avoid this error it was found necessary to evaporate all 155° and 165° distillates, and to redistil them below 145° C.

The effect of sulfates is shown by the comparison in Table 5.

<sup>1</sup> *This Journal*, 19, 316 (1930).

<sup>2</sup> Wichmann and Dahle, *This Journal*, 16, 612 (1933).

TABLE 5.—*Effect of sulfates*

| TEMPERATURE OF DISTILLATION | F RECOVERED (INDICATED) |                  |
|-----------------------------|-------------------------|------------------|
|                             | 1ST DISTILLATION        | 2ND DISTILLATION |
| °C.                         | per cent                | per cent         |
| 155                         | 51.5                    | 80.5             |
| 165                         | 52.5                    | 100.0            |

In the experiment a 300 cc. distillate was collected at each of the above temperatures. In 100 cc., fluorine was determined directly, while the remaining 200 cc. was made alkaline, evaporated, and redistilled at 135° C. As shown by the results errors as large as 47.5 per cent may be caused by the presence of sulfates.

#### 5. EFFECT OF VARYING OUTPUT VOLUME

The experimental conditions follow:

|                             |   |
|-----------------------------|---|
| <i>Temperature:</i>         | Constant at 130° C.   |
| <i>Input volume:</i>        | Constant at 25 cc.  |
| <i>Fluorine as NaF:</i>     | Constant at 0.5 mg.   |
| <i>Concentration of Al:</i> | Constant at 3 g. $\text{Al}_2(\text{SO}_4)_3$ 18 $\text{H}_2\text{O}$ . |
| <i>Output volume:</i>       | Varying.  |

The results are shown in Table 6 and Chart 4.

TABLE 6.—*Effect of varying output volume*

| OUTPUT VOLUME | F RECOVERED |            |
|---------------|-------------|------------|
|               | FOUND       | CALCULATED |
| cc.           | per cent    | per cent   |
| 100           | 41.2        | 42.4       |
| 200           | 61.2        | 66.8       |
| 300           | 83.6        | 82.2       |
| 500           | 95.0        | 93.4       |
| 1000          | 102.5       | 99.6       |

The recovery in the process varies with the output volume according to the logarithmic curve of a uni-molecular reaction. The "calculated recovery," represented by the broken line curve, was obtained by methods discussed in the previous publication.<sup>1</sup>

The recovery in a given amount of distillate should be independent of the time required for its collection. This factor was studied as follows:

|                       |                     |
|-----------------------|---------------------|
| <i>Input volume:</i>  | Constant at 22 cc.  |
| <i>Output volume:</i> | Constant at 200 cc. |

<sup>1</sup> *This Journal*, 19, 317 (1936)

|                              |   |
|------------------------------|---|
| <i>Temperature:</i>          | Constant at 135° C.   |
| <i>Fluorine as NaF:</i>      | Constant at 0.45 mg.  |
| <i>Concentration of Al:</i>  | Constant at 5 g. $\text{Al}_2(\text{SO}_4)_3 \cdot 18 \text{H}_2\text{O}$ . |
| <i>Time of Distillation:</i> | Varying.  |

The results are shown in Table 7.

## RESULTS

TABLE 7.—*Time rate vs. space rate*

| TIME | F ADDED | F RECOVERED |          |
|------|---------|-------------|----------|
| min. | mg.     | mg          | per cent |
| 30   | 0.45    | 0.3035      | 67.4     |
| 51   | 0.45    | 0.3030      | 67.3     |

The fact that the percentage recovery is independent of the original fluorine concentrations was also checked. The experimental conditions were the following:

Seven grams of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$  was dissolved in water, and 10 cc. of sulfuric acid and enough NaF to make the total amount of fluorine present equal 0.13 mg. were added. The mixture was distilled at  $147^\circ \text{C.} \pm 3^\circ$ , and 200 cc. of distillate was collected.

The experiment was then repeated under the same conditions, but the fluorine concentrations were equal to 0.53 mg. and 1.03 mg., respectively. The recoveries are shown in Table 8.

TABLE 8.—*Effect of fluorine concentration on recovery*

| F CONCENTRATION | F RECOVERED IN 200 cc |
|-----------------|-----------------------|
| mg' F.          | per cent              |
| 0.13            | 81.2                  |
| 0.53            | 80.2                  |
| 1.03            | 80.8                  |

## 6. APPLICATION

From Chart 3 it may be concluded that to get a complete separation of small quantities of fluorine from 0.8 gram of aluminum (10 grams  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ ) requires, where 10 cc. of sulfuric acid is used, the collecting of more than 200 cc. of distillate at a temperature between 160 and  $165^\circ \text{C}$ . One objection to this high temperature is the precipitation of anhydrous aluminum salts in the distilling flask. It was thought that perhaps an increased input volume, for example, the use of 15 cc. of sulfuric acid instead of 10 cc., might at least cut down, if it did not prevent, the precipitation to a point where distilling at  $165^\circ \text{C}$ . would become practicable.

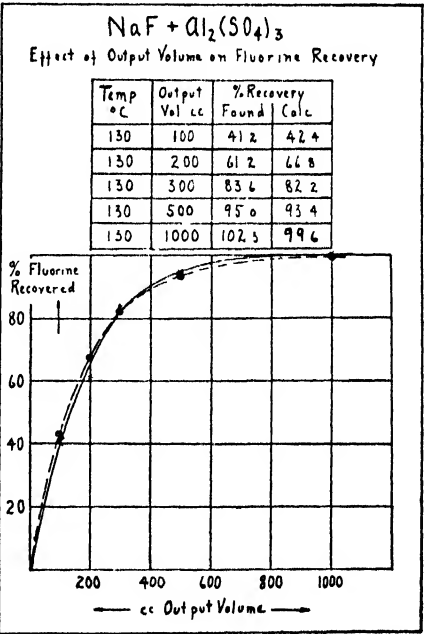
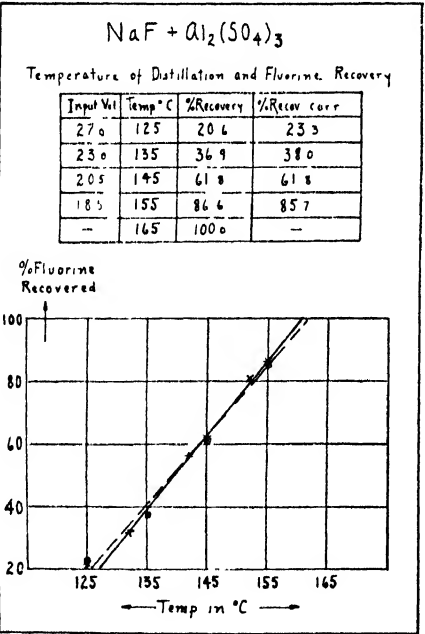
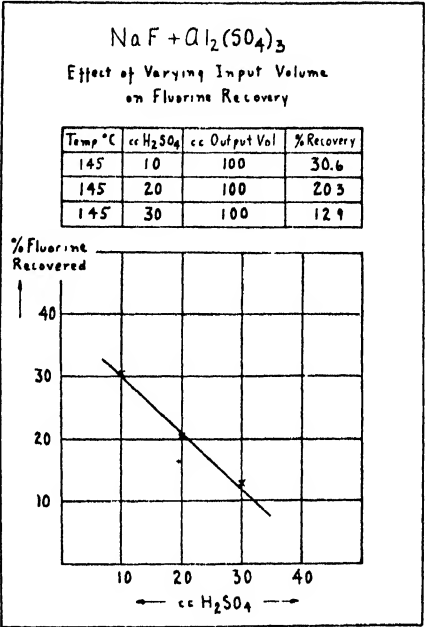
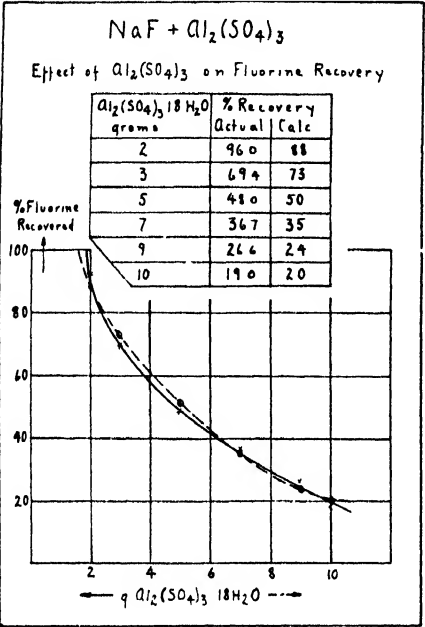


CHART 1  
CHART 3

CHART 2  
CHART 4

Chart 3 shows that at 145° C. there is a recovery of 63 per cent in 200 cc. when 10 cc. of sulfuric acid is used. This means 39 per cent in 100 cc.

$$\left( \frac{1}{200} \log \frac{100}{100-63} = \frac{1}{100} \log \frac{100}{100-X}; X=39 \right).$$

Chart 2 shows that approximately 5 per cent less, or 34 per cent, will be recovered if 15 cc. of the acid is used in the input; 34 per cent in 100 cc. output corresponds to 56.4 per cent in 200 cc. (see equation) at 145° C. Chart 3 also shows an increase in recovery of 2 per cent for each 1° C. rise in temperature. A 40 per cent increase may thus be gained by distilling at 165° C., and a total of 96.4 per cent may be recovered in 200 cc. output volume, or 99.1 per cent in 300 cc.

#### *Found Experimentally*

(1) With 10 grams of  $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ : No precipitate in the flask at 165° C. and a recovery of 99.8 per cent.

(2) With 7 grams of  $\text{Al}_2(\text{SO}_4)_3 \cdot \text{Na}_2\text{SO}_4 \cdot 24\text{H}_2\text{O}$  (equivalent in aluminum to 10 grams of aluminum sulfate): No precipitation and 100.5 per cent recovery.

Later experiments along the same line showed precipitates, but in no case enough to cause violent bumping or breakage of flasks.

Based on the foregoing experiments the following method may be suggested for determining fluorine in aluminum sulfate and alums:

#### METHOD

In a 125 cc. Claissen flask place an aliquot portion of sample containing not more than 0.8 gram of aluminum. Add 20 cc. of  $\text{H}_2\text{O}$  and 15 cc. of  $\text{H}_2\text{SO}_4$ . Connect for a Willard and Winter distillation, mix, heat carefully to boiling, and distil at 162° C.  $\pm 2^\circ$ . Collect 300-350 cc. of distillate at this temperature. Make alkaline to phenolphthalein, and evaporate in a porcelain casserole to about 50 cc. volume. Cool, and add 5 cc. of 30 per cent  $\text{H}_2\text{O}_2$ . Again evaporate, this time to about 5 cc. volume. Transfer to a 125 cc. Claissen flask, add 7.5 cc. of  $\text{H}_2\text{SO}_4$ , and distil at 137° C.  $\pm 5^\circ$ , collecting 100 cc. of distillate after the temperature has reached 132° C. Make to 150 cc. Determine fluorine by the peroxidized titanium method<sup>1</sup> on an aliquot containing not to exceed 0.05 mg. of fluorine.

NOTE: By the above method fluorine in aluminum sulfate and alums may be detected if present in quantities of about 2 p.p.m. If smaller amounts are concerned or greater accuracy required, two or more aliquots of the sample, each containing 0.8 gram of Al, may be subjected to the first distillation, the distillates combined and evaporated, and the second distillation made on the combined distillates.

#### SUMMARY

A study of the separation of small quantities of fluorine from large amounts of aluminum is presented. It was found that for complete separation by distillation the usual procedure is not satisfactory. Distillation at higher temperatures or the collection of increased amounts of distillate is necessary. The effect on fluorine recovery of various pertinent factors was also studied, and a method is suggested for isolation of fluorine occurring as an impurity in aluminum sulfate and alums.

<sup>1</sup> *This Journal*, 16, 612 (1933).

COMPOSITION AND PROPERTIES OF  
SUPERPHOSPHATE

## IV. FREE ACIDS IN FRESH SUPERPHOSPHATE

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In a previous paper<sup>1</sup> dealing with free acid in superphosphate it was shown (1) that with careful attention to details of manipulation ether and acetone extractions give concordant and accurate results for free acid in mixtures of monocalcium phosphate and aqueous phosphoric acid whose free acid-water ratios fall within the range usually encountered in commercial superphosphate, and (2) that the results obtained by the two extraction methods on cured commercial superphosphate (2-3 months old) usually agree very well. The data for superphosphate show that the results by acetone extraction are usually the higher and that the difference is greatest in the freshest materials. It was also noted that whereas small quantities of free fluorine acids are present in cured superphosphates, the amounts observed in fresh superphosphate are several times greater than in the cured materials when they come on the market. These considerations and the fact that in plant control, where the free acid determination is used extensively, the analysis is usually made on superphosphate less than one month old prompted the investigation reported herein. The investigation comprises a study of the change in the quantities of free acids with the age of the superphosphate.

## GENERAL CONSIDERATION OF FREE ACID

When phosphate rock is treated with sulfuric acid in the manufacture of superphosphate, the free acid in the mixture includes free acids of phosphorus, halogens, and sulfur. Some of the free acids are soon almost completely removed from the solution by precipitation or by volatilization, and others persist more or less definitely in the free condition. The number of acids present in the free condition depends largely on (1) the composition of the ingredients, (2) the process of manufacture, and (3) the age of the superphosphate. Acidulation with phosphoric acid does not greatly alter the situation.

Orthophosphoric acid is without question the predominant free acid in superphosphate. Small quantities of fluophosphoric acid,  $\text{H}_2\text{PO}_3\text{F}$ , may be present, particularly in fresh materials. Other phosphorus acids that should be mentioned in this connection, although lack of specific data makes an intelligent discussion of their role as free acid impossible at this time, are the phospho-acids of iron and aluminum,<sup>2</sup> for example,  $\text{H}_3\text{Fe}(\text{PO}_4)_2$ , and pyro- and even metaphosphoric acids in superphos-

<sup>1</sup> Hill and Beeson, *This Journal*, 18, 244 (1935).

<sup>2</sup> Santfourche, *Bull. soc. chim.*, (4) 53, 1517 (1933).

phate that has been dried at relatively high temperatures. The quantities of halogens other than fluorine in superphosphate are usually negligibly small. Only the simpler fluorine acids, HF,  $\text{H}_2\text{PO}_3\text{F}$ , and  $\text{H}_2\text{SiF}_6$ , are considered in this paper. Although sulfuric acid is the only acid of sulfur considered in later discussion, it should be pointed out that hydrosulfuric and sulfurous acids may be present in very fresh superphosphate—the former arising from the presence of sulfides in the natural phosphate, the latter from the reduction of sulfuric acid by organic matter and other reducing constituents of the rock.

At the time the ingredients of ordinary superphosphate are mixed the phosphoric acid ( $\text{H}_3\text{PO}_4$ ) equivalent of the total free acid ( $A$ ), in per cent by weight of the mixture is expressed with reasonable accuracy in the general case by the equation, (1)  $A = p + (k_1f_1 + k_2f_2 + \dots) + cp_s$ , in which  $p$  is the free phosphoric acid,  $f_1, f_2, \dots$  represent the fluorine present as the free fluorine acids, respectively,  $p_s$  is the free sulfuric acid, and  $k_1, k_2, \dots$ , and  $c$  are the appropriate conversion factors. A few hours after mixing  $p_s$  becomes very small, and accordingly it need not be considered in day-old superphosphate. Whereas several free fluorine acids may exist simultaneously, it is not unreasonable to suppose that some one predominates in any given case. Then, if the quantities of the other fluorine acids are small enough to be negligible in comparison with the predominant one, the equation becomes further simplified to (2)  $A = p + kF$ . When the total free acid undergoes change with the elapse of time, (3)  $\Delta A = \Delta p + k\Delta F$ , provided the value of  $k$  is not altered as a result of a shifting of the predominant fluorine acid. The change in free acid may be accompanied by various restrictions. The condition that the free phosphoric acid does not change ( $\Delta p = 0$ ) provides an important limiting case, which is approached more or less closely by a day-old superphosphate that contains only a very small percentage of citrate-insoluble  $\text{P}_2\text{O}_5$ . Then, in the limiting case (4)  $\Delta A = k\Delta F$ .

#### ANALYTICAL METHODS

The determination of the quantities  $A$ ,  $p$  and  $F$  in equation (2) is fraught with uncertainties arising from possible changes in the relative amounts of the free acids that may be caused by the analytical operations. However, two properly chosen methods of extracting the free acid can, as a result of a difference in the behavior of the fluorine acids with respect to the two extractants, give sets of results which, when they are correlated with the aid of equation (2), make possible certain important deductions concerning the nature and amounts of the free fluorine acids in superphosphate. Accordingly, for reasons which will become apparent from subsequent discussion, and until more satisfactory methods are developed, the result for free acid by acetone extraction will be regarded as the total free acid,  $A_a$ , the result by ether extraction (excluding the ex-



tracted fluorine prior to titration) will be taken as the free phosphoric acid,  $p_0$ , and the fluorine in the ether extract will be regarded as the fluorine equivalent of the free fluorine acids,  $F_0$ . Accordingly, for laboratory purposes equation (2) may be written thus: (5)  $A_0 = p_0 + kF_0 + r$ , in which  $r$  is the resultant effect of errors of manipulation ( $<0.1$  per cent of the sample) and of unknown, though possible, differences between the behavior with respect to the two extractants of readily decomposable complex acids and of certain minor metallic components of the superphosphate solution, such as magnesium and the alkalis.

The acetone extracts of superphosphate have been found to contain only about 20–40 per cent as much fluorine as do the ether extracts. In the case of two superphosphates that were several months old, the only materials for which data are available, the quantities of fluorine extracted by ether agreed reasonably well with those indicated by the analysis of superphosphate solutions<sup>1</sup> synthesized from the aforementioned superphosphates. Therefore, it appears that the difference between the quantities of fluorine found in the acetone and ether extracts results from the precipitation of fluorine acid during extraction with acetone rather than from the decomposition of fluorine-bearing solid constituents during extraction with ether. Accordingly, when the acetone method is used, a part of the free fluorine acid present in the superphosphate is precipitated during extraction; the other part goes into the extract and is subsequently titrated, in part at least, with the extracted phosphoric acid. As a result of the precipitation of a part of the fluorine acid an additional quantity of phosphoric acid appears in the acetone extract, which accounts for the experimental fact that in the case of material with relatively high percentages of free fluorine acids greater quantities of  $H_3PO_4$  are extracted by acetone than by ether. The value of  $k$  in equation (5) is not affected by this division of the fluorine acid, provided the equivalence factor for the fraction of the fluorine precipitated during extraction is identical with that for the part subject to titration. The titration characteristics of the common fluorine acids that may be present as free acid in superphosphate are given in Table 1.

The details of the analytical methods used by the writers are as follows:

*Total free acid (acetone method).*<sup>2</sup>—To 2 grams of the superphosphate (20-mesh) in a 100 ml. volumetric flask, add exactly 100 ml. of neutral C. P. acetone; stopper the flask, and mix the contents thoroughly at intervals of 5 minutes over a period of 2 hours. Filter the solution through a paper of medium porosity (for example, Whatman No. 40 filter paper), taking care to keep the paper well-filled with liquid and the funnel covered when possible during filtration. Taking more than the usual precaution to have the temperature of the extract the same as the temperature of the acetone when it was measured out, transfer at once a 50 ml. aliquot of the clear filtrate to a 400 ml. beaker containing 200 ml. of distilled water, add 4 drops of indicator (0.2 per cent solution of sodium alizarin sulfonate in water), and titrate

<sup>1</sup> Hill and Beeson, *Loc. cit.*

<sup>2</sup> Schucht, *Z. angew. Chem.*, 18, 1020 (1905); Shuey, *Ind. Eng. Chem.*, 17, 269 (1925).

TABLE 1.—*Titration characteristics of fluorine acids*  
(End point of sodium alizarin sulfonate, 25°–30° C.)

| ACID                                  | EQUATION OF TITRATION   | k <sup>a</sup> | REMARKS   |
|---------------------------------------|---|----------------|---|
| HF                                    | $\text{HF} + \text{NaOH} = \text{NaF} + \text{H}_2\text{O}$   | 5.16           | Equivalence point is not quite reached  |
| $\text{H}_2\text{SiF}_6$              | $\text{H}_2\text{SiF}_6 + x\text{NaOH} = y\text{Na}_2\text{SiF}_6 + z\text{NaF} + \dots$                    | 2.6            | $x=3$ approximately <sup>b</sup>  |
| $\text{H}_2\text{PO}_3\text{F}^\circ$ | $\text{H}_2\text{PO}_3\text{F} + \text{NaOH} = \text{NaHPO}_3\text{F} + \text{H}_2\text{O}$                 | 0.00           | $\text{H}_2\text{PO}_3\text{F}$ undergoes hydrolysis fairly rapidly, forming $\text{H}_2\text{PO}_4$ and $\text{HF}^\text{d}$ |
|                                       | $\text{H}_2\text{PO}_3\text{F} + 2\text{NaOH} = \text{NaH}_2\text{PO}_4 + \text{NaF} + 2\text{H}_2\text{O}$ | 5.16           |   |

<sup>a</sup> Factor for the conversion of the fluorine equivalent of the acid to  $\text{H}_2\text{PO}_4$ .

<sup>b</sup> Ross and Beeson, *This Journal*, 17, 238 (1934).

<sup>c</sup> Lange, *Ber.*, 62B, 793, 1084 (1929); Lange and Stein, *Ber.*, 64B, 2772 (1931), Lange, *Z. anorg. allgem. Chem.*, 214, 44 (1933).

<sup>d</sup> Lange, *Ber.*, 62B, 793 (1929).

with 0.1 *M* alkali until the color matches that of an equal volume of the same medium containing the same amount of indicator and an equivalent (approximately) quantity of pure monopotassium phosphate. Express the results of the titration in percentage of  $\text{H}_2\text{PO}_4$ .

*Free phosphoric acid.*—Extract 2.5 grams with 100 ml. of water-free ether as described in a previous paper,<sup>1</sup> dilute the extract to about 250 ml. with 60 per cent ethyl alcohol, and when the solution has cooled to room temperature make up to 250 ml. Evaporate an aliquot of 100 ml. of the homogeneous solution in a 250 ml beaker on a water bath, taking care to avoid violent boiling with consequent loss of acid in the spray, until the odor of alcohol has disappeared, and then allow to stand on the bath 15–30 minutes longer to expel the fluorine. Take up the fluorine-free phosphoric acid in about 25 ml. of water and pour through a small filter; wash the beaker and filter with cold water until the volume of the filtrate is 100 ml. Titrate this solution with 2 drops of the alizarin indicator as directed under *Total free acid*.

*Fluorine equivalent of the free fluorine acids.*—Make an aliquot of 50 ml. of the diluted ether extract of the superphosphate alkaline to phenolphthalein with dilute sodium hydroxide and then carefully evaporate to a volume of about 3 ml. Determine the fluorine in this concentrated solution by the method of Willard and Winter.<sup>2</sup>

#### MATERIALS

In the selection of materials for this study one of the main objectives was to obtain superphosphates that represented a wide range in composition with special reference to the presence or virtual absence of one or more of the components of phosphate rock other than phosphorus and calcium, viz.: F,  $\text{SiO}_2$ ,  $\text{R}_2\text{O}_3$ ,  $\text{MgO}$ , and the alkalis. Whereas about 30 different samples were used, the composition range is represented by the eight ordinary superphosphates described in Table 2, comprising seven materials prepared in the laboratory by a method described previously<sup>3</sup> and one superphosphate prepared commercially.

<sup>1</sup> Hill and Jacob, *This Journal*, 17, 487 (1934).

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 3, 7 (1933).

<sup>3</sup> Hill and Hendricks, *Ind. Eng. Chem.*, 28, 440 (1936).

TABLE 2.—Composition of day-old ordinary superphosphates

| SAMPLE            | SUPERPHOSPHATE MADE FROM—                                      | P <sub>2</sub> O <sub>5</sub> |                   |                        |                    | F            | SiO <sub>2</sub> <sup>b</sup> | Al <sub>2</sub> O <sub>3</sub> | Fe <sub>2</sub> O <sub>3</sub> | MgO          | Na <sub>2</sub> O+K <sub>2</sub> O | H <sub>2</sub> O  |          |
|-------------------|--|-------------------------------|-------------------|------------------------|--------------------|--------------|-------------------------------|--------------------------------|--------------------------------|--------------|------------------------------------|-------------------|----------|
|                   |  | TOTAL                         | CITRATE-INSOLUBLE | FREE ACID <sup>a</sup> | CRYSTALLIZATION    |              |                               |                                |                                |              |                                    | FREE <sup>a</sup> |          |
|                   |  |                               |                   |                        |                    |              |                               |                                |                                |              |                                    |                   | per cent |
| ES-11             | C. P. tricalcium phosphate                                     | 27.90                         | 0.00              | 2.82                   | —                  | —            | —                             | —                              | —                              | —            | —                                  | 3.15              | 7.90     |
| ES-5              | Bone ash No. 1225  | 22.09                         | 0.51              | 5.33                   | <0.03 <sup>b</sup> | 0.2          | 0.00                          | 0.10                           | 0.60                           | 0.51         | —                                  | 2.31              | 7.47     |
| ES-10             | Bone ash No. 1225 + R <sub>2</sub> O <sub>3</sub> <sup>c</sup> | 20.80                         | 0.15              | 6.14                   | <0.03 <sup>b</sup> | 0.2          | 0.44                          | 1.45                           | 0.55                           | 0.46         | —                                  | 2.58              | 10.03    |
| ES-8              | Nauru Island phosphate   | 20.74                         | 0.28              | 7.77                   | 1.34               | 0.1          | <0.15                         | •                              | 0.00                           | 0.24         | —                                  | 2.97              | 10.43    |
| ES-9              | Nauru Island phosphate   | 20.10                         | 0.29              | 7.55                   | 1.30               | 2.5          | <0.15                         | •                              | 0.00                           | 0.23         | —                                  | 2.96              | 9.80     |
| ES-3              | No. 450+SiO <sub>2</sub> <sup>f</sup>                          | 18.98                         | 0.18              | 7.58                   | 1.61               | 4.0          | 0.56                          | 0.38                           | 0.04                           | 0.14         | —                                  | 1.81              | 9.93     |
| 1423 <sup>g</sup> | Florida land pebble  | 18.69                         | 3.01              | 6.01                   | 1.68               | <sup>b</sup> | 0.21                          | 1.07                           | <sup>b</sup>                   | <sup>b</sup> | —                                  | 1.63              | 11.59    |
| ES-4              | Tennessee brown rock   | 18.45                         | 0.23              | 7.88                   | 1.46               | 3.1          | 0.62                          | 1.35                           | 0.01                           | 0.24         | —                                  | 1.77              | 10.19    |
|                   | No. 908 <sup>d</sup>   |                               |                   |                        |                    |              |                               |                                |                                |              |                                    |                   |          |

<sup>a</sup> Determined by extraction with ether; result does not include extracted fluorine.<sup>b</sup> Calculated from the composition of the original phosphate material. Loss by volatilization disregarded.<sup>c</sup> Natural aluminum phosphate (sample 904) equivalent to 1.25% of Al<sub>2</sub>O<sub>3</sub>, and sufficient C. P. Fe<sub>2</sub>O<sub>3</sub> to bring the total iron (Fe<sub>2</sub>O<sub>3</sub>) to 2.5% of the unacidulated phosphate mixture.<sup>d</sup> Complete analysis given by Jacob et al, U. S. Dept. Agr. Tech. Bull. 364 (1933).<sup>e</sup> Included in figure for Al<sub>2</sub>O<sub>3</sub>.<sup>f</sup> Quarts flour (100-mesh).<sup>g</sup> Commercial material.<sup>h</sup> Not determined.

When the laboratory materials were 1 day old they were ground to pass a 20-mesh sieve and stored in tightly stoppered bottles. The day-old commercial superphosphate was put through a 10-mesh sieve and kept in a tightly stoppered bottle. For the periodic determination of the free acid a sample representative of the entire bottle of 10-mesh material (fresh sample taken each time by quartering) was ground to pass a 20-mesh sieve. A portion of the 10-mesh commercial material was stored at a temperature of 60°-70° C. in a glass container provided with a small opening for the escape of gases; it was allowed to cool before the analytical sample was taken. The other materials were kept at 25°-30° C.

#### CHANGE IN FREE ACID WITH THE AGE OF SUPERPHOSPHATE

*Fluorine-free superphosphate.*—The materials representing this type of superphosphate are ES-11, ES-5, and ES-10, made from C P. tricalcium phosphate, bone ash, and bone ash with additions of aluminum and iron compounds, respectively. Plots showing the effect of age on the free acid (results by ether extraction method) in superphosphate prepared from tricalcium phosphate and bone ash, respectively, were given in a previous paper;<sup>1</sup> therefore, curves for such materials need not be reproduced here.

It is to be supposed that in day-old superphosphate that does not contain fluorine the total free acid is equal to the free phosphoric acid. Accordingly, the results for free acid obtained by ether and acetone extractions should agree within the limits of analytical error. The experimental results show almost perfect agreement (0.1 per cent or less) in the case of superphosphate prepared from tricalcium phosphate. On the other hand, the results obtained on bone-ash superphosphate differed by as much as 2 per cent; moreover acetone extraction always gave the lower figures. As an example illustrating this condition, results obtained on a superphosphate made from a mixture of bone ash with aluminum and iron compounds are given in Table 3. It may be noted in this connection

TABLE 3.—*Effect of age on the free acid in bone-ash superphosphate ES-10*  
(Results expressed in percentage of day-old material)

| AGE OF SUPERPHOSPHATE | FREE ACID BY EXTRACTION WITH— |       | DIFFERENCE |
|-----------------------|-------------------------------|-------|------------|
|                       | ACETONE                       | ETHER |            |
| <i>days</i>           |                               |       |            |
| 1                     | 6.64                          | 8.47  | -1.83      |
| 10                    | 5.61                          | 7.63  | -2.02      |
| 30                    | 5.27                          | 6.30  | -1.03      |
| 90                    | 5.64                          | 5.81  | -0.17      |

that with bone superphosphate that contained only small quantities of  $R_2O_3$  (for example ES-5) the results by the two extraction methods converge somewhat more slowly than in the case for which data are given.

<sup>1</sup> Hill and Beeson, *Loc. cit.*

This disparity between the results by extraction with acetone and with ether was thought to be attributable to differences in the behavior of magnesium or alkali phosphates towards the extractants, but results obtained on comparable synthetic mixtures (Table 4) do not support this view. The writers have found no satisfactory explanation for the apparent irregularity of the results for the bone-ash superphosphates.

TABLE 4.—*Effect of magnesium and alkali phosphates on the determination of free acid by extraction with ether and with acetone*  
(Results expressed in percentage of added  $H_3PO_4$ )

| MIXTURE <sup>a</sup> COMPRISING 9 PARTS<br>OF $Ca(H_2PO_4)_2 \cdot H_2O$ AND 1<br>PART OF — | FREE ACID BY EXTRACTION WITH— |       | DIFFERENCE |
|---|-------------------------------|-------|------------|
|   | ACETONE                       | ETHER |            |
| $Mg(H_2PO_4)_2$   | 108                           | 104   | +4         |
| $NaH_2PO_4$   | 100                           | 98    | +2         |
| $KH_2PO_4$  | 102                           | 97    | +5         |
| $MgHPO_4$   | 73                            | 69    | +4         |
| $Na_2HPO_4$   | 70                            | 66    | +4         |

<sup>a</sup> To 1 gram of the acid-free mixture was added a known quantity of 42.5% aqueous phosphoric acid sufficient to yield a synthetic sample that contained 8–9% of added  $H_3PO_4$ .

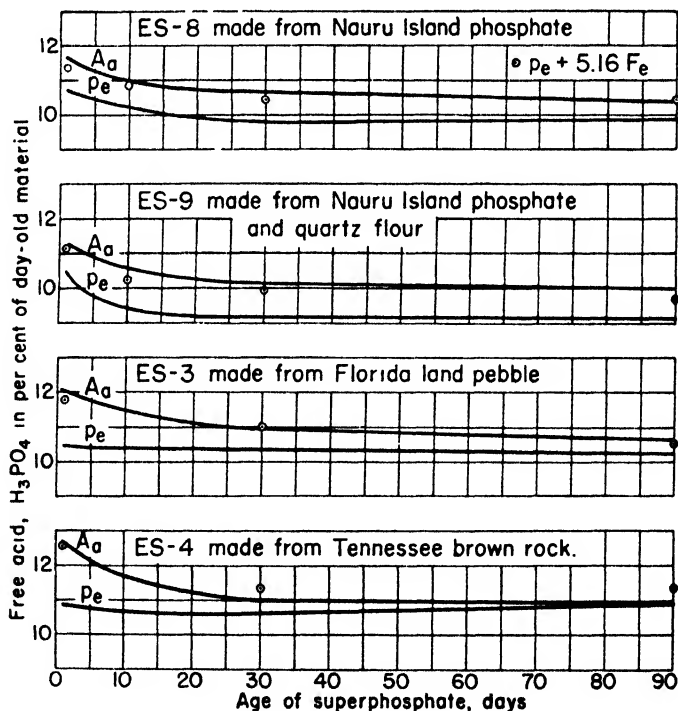


FIG. 1.—EFFECT OF AGE ON THE AMOUNTS OF TOTAL FREE ACID ( $A_a$ ) AND FREE PHOSPHORIC ACID ( $p_e$ ) IN ORDINARY SUPERPHOSPHATE PREPARED IN THE LABORATORY AND STORED AT 25°–30° C.

*Fluorine-bearing superphosphate.*—The effect of age on the quantities of total free acid and free phosphoric acid in the superphosphates prepared in the laboratory and stored at 25°–30° C. is shown in Fig. 1 with the aid of curves obtained by drawing a smooth line through the points (not shown in the plot) representing the percentage of free acid when the superphosphate was 1, 10, 30, and 90 days old, respectively. Similar curves are given in Fig. 2 for the commercial superphosphate.

It will be noted (Table 2) that the laboratory-prepared materials were initially very low in citrate-insoluble phosphorus (0.3 per cent of  $P_2O_5$  or less), whereas the commercial superphosphate contained 3.01 per cent. Accordingly, the latter material would be expected to show the greater change in free acid with age. Furthermore, the citrate-insoluble phosphorus in ES-8 and ES-9 dropped in 30 days to 0.07 and 0.03 per cent, respectively, whereas it was sensibly constant in ES-3 and ES-4 over the 90-day period. When allowance is made for initial differences in citrate-insoluble phosphorus, a marked similarity is recognized in all pairs of curves. The curve for total free acid always lies above and with increase in age approaches that for free phosphoric acid. The total free acid always decreases, even in those cases where the amount of free phosphoric

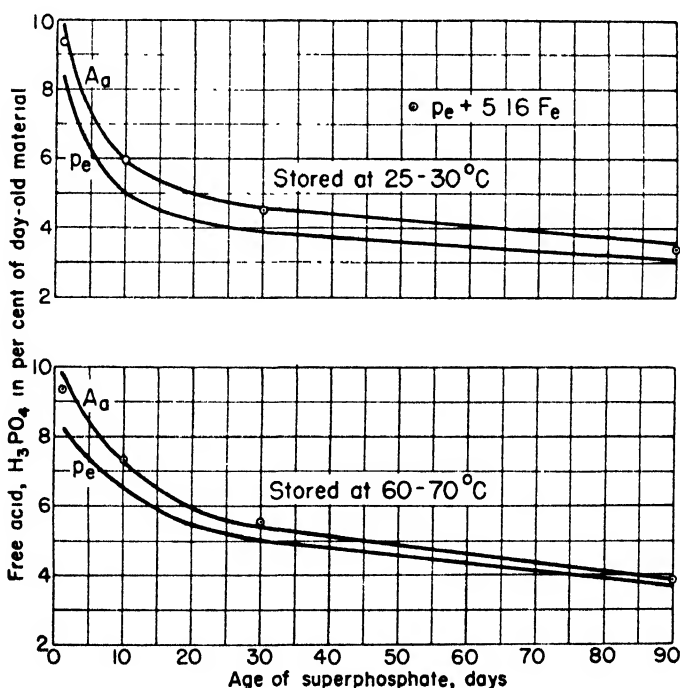


FIG. 2.—EFFECT OF AGE ON THE AMOUNTS OF TOTAL FREE ACID ( $A_a$ ) AND FREE PHOSPHORIC ACID ( $p_e$ ) IN ORDINARY SUPERPHOSPHATE PREPARED COMMERCIALY.

acid remains practically constant or shows a slight increase (see equation 4). Important differences between the pairs of curves are to be noted in the spacing and the rate at which the lines converge.

The change in the total amount of free fluorine acids with the age of the superphosphate (Fig. 3) in most cases shows the same trend as the change in total free acid (Figs. 1 and 2). With the exception of the  $R_2O_3$ -free (nearly) superphosphates (ES-8 and 9) prepared from Nauru Island phosphate, the curves show a regular decrease in the quantity of fluorine

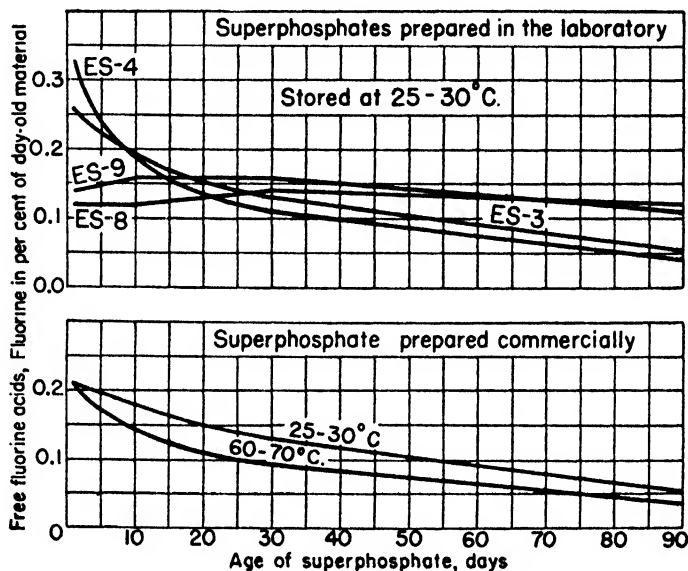


FIG. 3.—EFFECT OF AGE ON THE TOTAL AMOUNT (FLUORINE EQUIVALENT,  $F_e$ ) OF FREE FLUORINE ACIDS IN ORDINARY SUPERPHOSPHATE

acids. Since observable changes in the total amount of fluorine did not occur in any of the materials, the decrease in the quantity of fluorine acid cannot be attributed to loss of fluorine by volatilization. It will also be observed that in conformity with equation (3) the rate of decrease of the free fluorine acids bears some relation to the rate of convergence of the curves for total free acid and free phosphoric acid.

#### FREE FLUORINE ACID IN SUPERPHOSPHATE

In accordance with equation (5) the distance between the curves for total free acid and free phosphoric acid (Figs. 1 and 2) at a given age represents the magnitude of the sum  $kF_e + r$ . Since the values of  $F_e$  are now known (Fig. 3), it is possible to choose a fluorine acid (Table 1) whose  $k$ -value will yield the best agreement between the calculated values of  $kF_e$  and the differences between the determined percentages of total free

acid and free phosphoric acid. The relationship can be more conveniently observed on the plots by comparing the calculated value of the total free acid,  $p_s + kF_s$ , with the observed value,  $A_s$ . Accordingly, the fluorine acid with the highest  $k$  value, HF, was selected, and the values of the sum,  $p_s + 5.16 F_s$ , are indicated by encircled points in Figs. 1 and 2.

In most cases the agreement between the calculated and observed percentages for the phosphoric equivalent of the total free acid is close. The inference appears to be that hydrofluoric acid is the predominant free fluorine acid in superphosphate. Moreover, this view is supported by the fact that in the case of the superphosphates prepared from Nauru Island phosphate the silica-rich material showed about the same amounts of free fluorine acid as the one which was practically silica-free. The negligible effect of the presence of silica observed in this instance seems to indicate that no more than a small fraction of the free-acid fluorine in superphosphate exists as free hydrofluosilicic acid. Furthermore, on the basis of equilibrium measurements<sup>1</sup> for the reaction,  $H_3PO_4 + HF = H_2PO_3F + H_2O$ , only small quantities of free fluophosphoric acid would be expected.

#### EFFECT OF TEMPERATURE ON THE CHANGE IN FREE ACID WITH AGE

In the early stages of the writers' work on superphosphate the observation was made that commercial materials, as they come on the market carry smaller quantities of free fluorine acids than do materials of approximately the same age that had been prepared and cured in the laboratory. In commercial practice, where the superphosphate is usually cured in large piles, the temperature within the pile remains relatively high over a considerable period of time, whereas the laboratory-prepared materials were cooled to room temperature at the end of the first day. On this basis the smaller quantities of free fluorine acid in the commercial materials was attributed to the higher temperature that in all probability prevailed during curing, and in order to test the validity of this conclusion comparable data at two temperatures were obtained on a Florida pebble superphosphate. Losses of fluorine by volatilization during the experiments were not detectable by analyses of the material under test. The results (Fig. 3) show that the higher temperature does favor the disappearance of free fluorine acid. On the other hand, the quantities of free phosphoric acid, and also of total free acid (Fig. 4) were greater in the material that was kept at the higher temperature.

#### SUMMARY

Previous work on free acid in superphosphate has been extended to include fresh material.

<sup>1</sup> Lange, *Ber.*, 62B, 1084 (1929).



The acids that do, or may, occur in the free condition in superphosphate are discussed, and a simple equation is formulated to express the relationship between the total free acid on the one hand and the free phosphoric acid and the predominant free fluorine acid on the other.

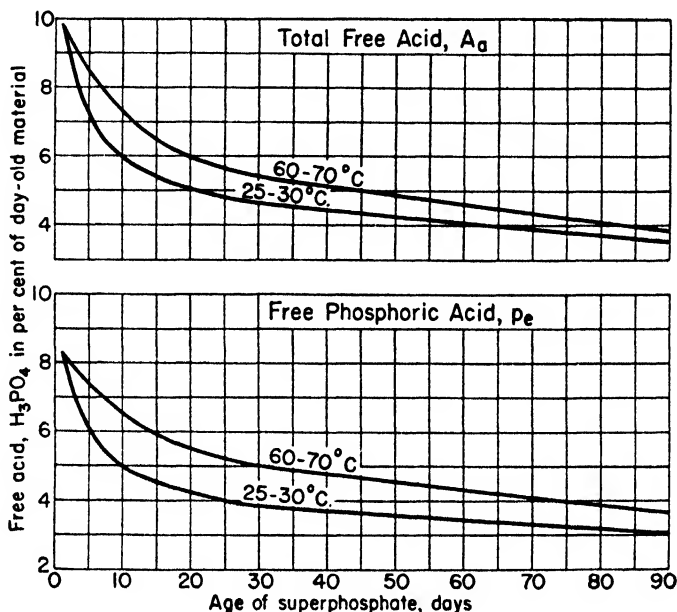


FIG. 4.—EFFECT OF TEMPERATURE OF CURING ON THE AMOUNT OF FREE ACID IN COMMERCIAL FLORIDA LAND-PEBBLE SUPERPHOSPHATE

Methods for determining the quantities entering the equation are discussed, and results are given to show the effect of (1) age of the material and (2) temperature of curing on the amounts of free acids in superphosphates.

Correlation of the data leaves the inference that hydrofluoric acid is the predominant free fluorine acid in fresh superphosphate.

### SOME OBSERVATIONS RELATIVE TO DETECTING THE ADULTERATION OF HONEY WITH COMMERCIAL INVERT SUGAR\*

By R. E. LOTHROP (Carbohydrate Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

Present methods of detecting the adulteration of honey with commercial invert sugar are not always satisfactory. Both of these products

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, November, 1935.

contain similar relative proportions of various sugars, and the addition of even large quantities of commercial invert sugar may not alter the proportions of the sugars in the honey to any appreciable extent. The control chemist, therefore, must rely largely on indirect methods, such as the resorcinol test and the aniline chloride test,<sup>1</sup> for detecting this type of adulteration.

A positive response of a sample of honey to these tests indicates the presence of commercial invert sugar. However, the tests are by no means infallible, since under certain conditions genuine honey shows a positive reaction. For example, after honey is heated, sufficient furfural derivatives may be formed to give positive color reactions, and after storage for long periods (five years or more) most honey gives positive resorcinol and aniline chloride tests. A combination of heating and storage results in positive color reactions in a shorter time than does storage alone.<sup>2</sup>

While, in experienced hands, these tests are undoubtedly of considerable value for detecting added commercial invert sugar, their value is limited. Moreover, the adulteration of honey with commercial invert sugar prepared with invertase in place of acid cannot be detected by the resorcinol and aniline chloride tests, since no furfural derivatives are formed during the inversion of sucrose with invertase. It is even possible by careful control of temperature, concentration of acid, and duration of heating, to prepare commercial invert sugar by acid inversion without formation of appreciable quantities of furfural derivatives.

It is apparent, therefore, that other independent means of detecting adulteration of honey with commercial invert sugar should be sought. Although the color tests may give direct evidence of the presence of commercial invert sugar in honey, to be conclusive they should be supported by additional evidence.

Auerbach and Bodlander<sup>3</sup> suggest the use of the levulose-dextrose ratio to distinguish between genuine and adulterated honeys, the assumption being that genuine honey always contains an excess of levulose over dextrose and that the range of this ratio lies between comparatively narrow limits. The addition of any appreciable quantity of commercial invert sugar, which usually contains slightly more dextrose than levulose when made by the usual methods of manufacture, would alter the ratio of levulose to dextrose in the honey sufficiently to bring it outside the range for genuine honey. However, this method would not be applicable to American honeys except to detect gross adulteration, as the ratio of levulose to dextrose ranges from almost 2:1 for tupelo honey to about 1:1 for such honeys as alfalfa.

Except for a few data given by Browne<sup>4</sup> there is practically no informa-

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 390.

<sup>2</sup> H. W. de Boer, *Chem. Weekblad*, 31, No. 3, 482-7 (1934).

<sup>3</sup> *Z. Nahr. Genussm.*, 47, 233 (1924).

<sup>4</sup> U. S. Bur. Chem. Bull. 110 (1908).

tion available on the nitrogen content of American honeys. In an investigation of the colloidal constituents of honey conducted by this Laboratory some time ago, a study of the nitrogenous constituents of American honeys was made. This study included a careful determination of the total nitrogen content of all the representative floral types of American honeys. The nitrogen content of the thirty-one samples ranged from 0.017 per cent for fireweed honey to 0.186 per cent for buckwheat, with an average value of 0.048 per cent for all the samples. Browne obtained an average value of 0.053 per cent for a smaller number of samples. The values are summarized in Table 1.

TABLE 1.—*Relation of color to the nitrogen and ash contents of American honeys*

| SAMPLE NO. | PREDOMINANT FLORAL TYPE | U. S. STANDARD COLOR GRADE (PFUND SCALE) |                   | NITROGEN CONTENT | ASH CONTENT | AVERAGE |
|------------|-------------------------|--|-------------------|------------------|-------------|---------|
|            |                         | READING                                  | CLASSIFICATION    |                  |             |         |
|            |                         |  |                   | per cent         | per cent    |         |
| 1251       | Fireweed                | 0.0                                      | Water white       | —                | 0.05        |         |
| 1229       | Sweet clover            | 0.2                                      | "                 | 0.017            | 0.07        |         |
| 1207       | Orange                  | 0.8                                      | "                 | 0.026            | 0.08        |         |
| 1285       | Fireweed                | 1.0                                      | Extra white       | 0.019            | 0.06        |         |
| 1234       | White sage              | 1.3                                      | "                 | 0.036            | 0.10        |         |
| 1201       | Catsclaw                | 1.5                                      | "                 | 0.031            | 0.11        |         |
| 1203       | Gallberry               | 1.5                                      | "                 | 0.032            | 0.15        |         |
| 1210       | Manzanita               | 1.6                                      | "                 | 0.028            | 0.31        |         |
| 1252       | Mesquite                | 1.6                                      | "                 | 0.034            | 0.11        |         |
| 1279       | White clover            | 1.7                                      | White             | 0.046            | 0.10        | 0.114   |
| 1206       | Catsclaw                | 1.9                                      | "                 | 0.048            | 0.10        | 0.0317  |
| 1248       | Sourwood                | 2.3                                      | "                 | 0.036            | 0.27        |         |
| 1244       | Mangrove                | 2.4                                      | "                 | 0.028            | 0.19        |         |
| 1216       | Tupelo                  | 3.3                                      | "                 | 0.031            | 0.09        |         |
| 1377       | Cotton                  | 3.5                                      | Extra Light Amber | 0.035            | 0.43        |         |
| 1255       | Wild flowers            | 3.7                                      | "                 | 0.046            | 0.14        |         |
| 1189       | Sumac                   | 3.9                                      | "                 | 0.047            | 0.33        |         |
| 1195       | Dandelion               | 4.4                                      | "                 | 0.039            | 0.39        |         |
| 1209       | Holly                   | 4.4                                      | "                 | 0.047            | 0.51        |         |
| 1213       | Eucalyptus              | 4.5                                      | "                 | 0.039            | 0.24        | 0.269   |
| 1214       | Fruit bloom             | 4.7                                      | "                 | 0.062            | 0.32        | 0.0410  |
| 1256       | Horse chestnut          | 5.1                                      | Light Amber       | 0.117            | 0.26        |         |
| 1208       | Tarweed                 | 5.6                                      | "                 | 0.052            | 0.60        |         |
| 1237       | Rosinweed               | 5.8                                      | "                 | 0.043            | 0.07        |         |
| 1287       | Bitterweed              | 5.8                                      | "                 | 0.037            | 0.16        |         |
| 1239       | Alfalfa                 | 6.0                                      | "                 | 0.046            | 0.13        |         |
| 1202       | Chinquapin              | 6.8                                      | "                 | 0.063            | 0.79        |         |
| 1257       | Wild flowers            | 8.3                                      | "                 | 0.053            | 0.40        |         |
| 1337       | Palmetto                | 8.3                                      | "                 | 0.049            | 0.23        |         |
| 1215       | Unknown source          | 11.5                                     | Dark              | 0.052            | 0.51        |         |
| 1184       | Buckwheat               | 12.9                                     | "                 | 0.186            | 0.13        | 0.327   |
| Average    |                         |  |                   | 0.048            | 0.239       |         |

Although many individual exceptions occur, in a general way the nitrogen content increases with increasing color of the honey. This will be evident if average values of the 10 lightest colored honeys are compared with average values for the next 10 samples and these in turn are compared with average values for the last 10, or darkest, samples. A somewhat similar relationship appears to exist between the color and ash content of honey.

Use of criteria such as the nitrogen and ash content for detecting adulteration is of greatest value when there is gross adulteration with commercial invert sugar. They are of little value when used without the supporting evidence of positive color reactions, or for detecting the addition to honey of relatively small proportions of commercial invert sugar, because in this case the contents of nitrogen and ash are not lowered appreciably. However, with honey suspected of being adulterated, very low nitrogen and ash values in addition to positive color tests are considered quite conclusive proof of adulteration. If the nitrogen or ash content (or both) falls below the minimum values for honey, this fact alone should be sufficient to prove adulteration.

It should also be pointed out that isolation of the acid used as the inverting agent in preparing the commercial invert sugar has been used as a confirmatory test of adulteration.<sup>1</sup> Since the most convenient method for preparing small batches of commercial invert sugar includes the use of tartaric acid, this acid, as well as hydrochloric or phosphoric, should be looked for. It is quite unlikely that invertase would be used as an inverting agent for preparing invert sugar for this purpose.

## STUDIES ON THE QUANTITATIVE ESTIMATION OF LIGNIN\*

### I. FACTORS AFFECTING THE DETERMINATION BY THE FUMING HYDROCHLORIC ACID METHOD

By M. J. Goss and MAX PHILLIPS

In a previous paper Phillips<sup>2</sup> describes a method for the quantitative estimation of lignin. As further studies showed that certain factors, unless carefully controlled, could seriously affect the results obtained by this method, it seemed advisable to study it further with a view to (1) ascertaining the effect of temperature, time of reaction, etc., on the yield and purity of lignin obtained, and (2) determining the optimum conditions for carrying out the determination. The results of such a study are presented in this paper.

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<sup>1</sup> Notice of Judgment No. 3406, Food and Drugs Act, Sept. 24, 1914.

<sup>2</sup> Contribution from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils U. S. Department of Agriculture, Washington, D. C.

<sup>3</sup> *This Journal*, 15, 118 (1932).

## EXPERIMENTAL

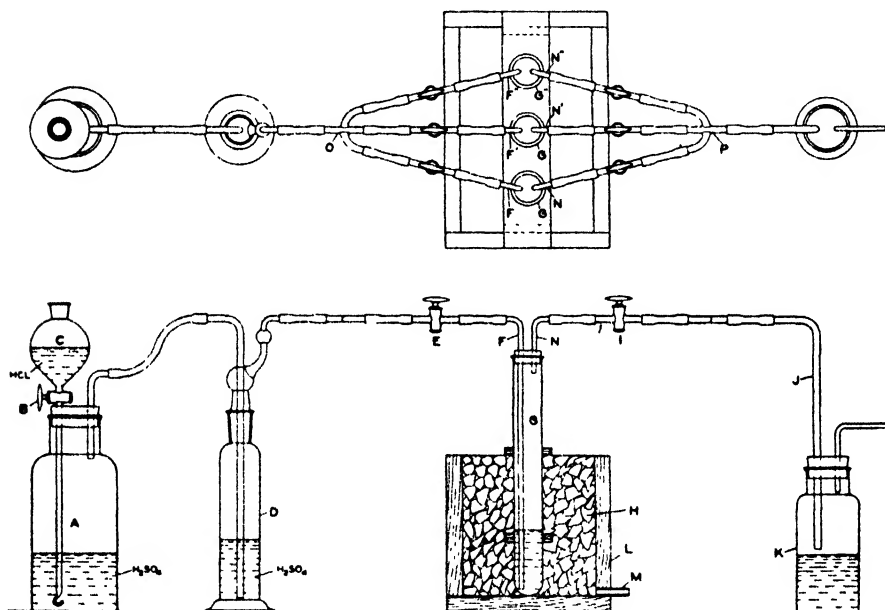
Wheat straw was the material used. It was ground to pass through an 80-mesh sieve. The material was extracted with 1:2 alcohol-benzene solution, hot water, and one per cent hydrochloric acid solution as directed by Phillips in *This Journal*, 18, 386 (1935). The preparation of the fuming hydrochloric acid used was described by Phillips in *This Journal*, 15, 118 (1932), and the apparatus used is described later in this paper. In some of the experiments the wheat straw had been extracted only with an alcohol-benzene solution, and in others with an alcohol-benzene solution followed by an extraction with hot water. These materials are indicated in the tables.

## ANALYTICAL EXPERIMENTS

*Effect of time of boiling with dilute hydrochloric acid on yield and purity of lignin.*—In the determination of lignin by the method previously referred to, the plant material is first subjected to the action of fuming hydrochloric acid in the cold and then to boiling dilute hydrochloric acid in order to convert the carbohydrates and associated substances into water-soluble products. In the first series of experiments, the effect of time of boiling with the dilute acid was determined. It was quite apparent that if the reaction period were relatively short the carbohydrates would not be completely hydrolyzed and high lignin values would be obtained. On the other hand, if the reaction period were very long, there would be the possibility that some of the lignin might be lost. In all experiments the methoxyl content of the lignin was used as a criterion of the purity of the product. Lignin contaminated with carbohydrates would necessarily give a low percentage of methoxyl.

The experiments to determine what effect time of heating with dilute acid had on the yield and purity of the lignin obtained were conducted as follows: Three one gram samples of the ground wheat straw that had been successively extracted with an alcohol-benzene solution, hot water, and one per cent hydrochloric acid solution were weighed out and placed in tubes G, G', and G'', 20 cc. of the fuming hydrochloric acid was added to each tube, care being taken to wash down with this acid any particles clinging to the sides. When all the material was wetted with the hydrochloric acid, another portion (30 cc.) of fuming hydrochloric acid was added. About three drops of capryl alcohol were also added. The tubes G, G', and G'' were placed in the wooden box L and surrounded with crushed ice. Tubes F, F' and F'' were lubricated with a little glycerol, and dry hydrochloric acid gas from the generator (A) was led into the reaction mixture through these tubes. The passage of the gas through the reaction mixture was continued for 2 hours. The tubes F, F', and F'' and the outlet tubes N, N' and N'' were disconnected from O and P. The tubes F, F', and F'' were pulled up just above the surface of the

reaction mixture, and these tubes as well as the outlet tubes N, N' and N'' were closed off. The tubes containing the reaction mixture were then placed in large beakers, packed with crushed ice, and allowed to remain in an ice box for 24 hours. The contents of tubes G, G', and G'' were then transferred to one liter Erlenmeyer flasks and diluted with water to a volume of 500 cc., and the flasks were connected to reflux condensers and boiled for varying periods (Table 1). The reaction mixtures were allowed to cool to room temperature, filtered into weighed



APPARATUS FOR QUANTITATIVE ESTIMATION OF LIGNIN

crucibles, and the percentage of lignin was determined following the procedure described by Phillips. The percentage of methoxyl in the lignin was determined either by the Kirpal and Bühn<sup>1</sup> modification of the Zeisel method<sup>2</sup> or by the semi-micro method of Vieböck and Schwappach.<sup>3</sup> However, the quantity of bromine recommended by Vieböck and Schwappach was found to be insufficient. About twenty drops of bromine were used for each 20–30 mg. sample. The lignin sample to be used for the methoxyl determination was collected in a sintered-glass crucible instead of in a Gooch crucible.

Table 1, Part I, shows that hydrolysis of the various substances associated with the lignin was complete after one hour of boiling. The per-

<sup>1</sup> *Ber.*, 47, 1084 (1914); *Monatsh.*, 36, 853 (1915).

<sup>2</sup> *Monatsh.*, 6, 989 (1885); 7 406 (1886).

<sup>3</sup> *Ber.*, 63, 2818 (1930).

TABLE 1.—*Lignin in wheat straw—effect of time of heating with dilute acid*

I. This straw was extracted successively with alcohol-benzene solution, hot water, and 1% HCl solution, loss due to extraction being 45.2%. All results calculated on original unextracted straw.

| EXPT. NO. | TIME OF HEATING<br>AT BOILING TEMP.<br>OF SOLUTION | WEIGHT OF SAMPLES |        | N IN<br>CRUDE LIGNIN | ASH IN<br>CRUDE LIGNIN |        | WEIGHT OF LIGNIN<br>(CORRECTED FOR ASH AND<br>CRUDE PROTEIN) |          | LIGNIN (CALCULATED ON<br>ORIGINAL DRY MATERIAL) |       | METHOXYLIN<br>ASH-FREE<br>CRUDE LIGNIN |
|-----------|--|-------------------|--------|----------------------|------------------------|--------|--|----------|---|-------|--|
|           |  | (a)               | (b)    |                      | (a)                    | (b)    | (a)  | (b)      | (a)   | (b)   |  |
|           | hours  | gram              |        | per cent             | per cent               | gram   |  | per cent | per cent  |       |  |
| 1         | 1  | 1.0000*           | 1.0000 | 0.72                 | 5.30                   | 0.2131 | 0.2127   | 11.67    | 11.65   | 15.41 |  |
| 2         | 2  | 1.0000            | 1.0000 | 0.58                 | 5.03                   | 0.2104 | 0.2087   | 11.53    | 11.43   | 15.50 |  |
| 3         | 4  | 1.0000            | 1.0000 | 0.61                 | 5.46                   | 0.2086 | 0.2091   | 11.43    | 11.45   | 15.47 |  |
| 4         | 6  | 1.0000            | 1.0000 | 0.51                 | 5.35                   | 0.2070 | 0.2085   | 11.34    | 11.42   | 15.51 |  |
| 5         | 8  | 1.0000            | 1.0000 | 0.50                 | 4.97                   | 0.2062 | 0.2049   | 11.30    | 11.22   | 15.45 |  |
| 6         | 10   | 1.0000            | 1.0000 | 0.51                 | 5.05                   | 0.2079 | 0.2051   | 11.39    | 11.23   | 15.31 |  |

II. This straw was extracted with alcohol-benzene solution, loss due to extraction being 6.00%. All results calculated on original unextracted straw.

|   |    |         |        | per cent | per cent |  |        |        | per cent | per cent |       |  |
|---|----|---------|--------|----------|----------|--|--------|--------|----------|----------|-------|--|
| 1 | 1  | 1.0000† | 1.0000 | 0.32     | 13.91    |  | 0.1439 | 0.1454 | 13.52    | 13.66    | 15.06 |  |
| 2 | 2  | 1.0000  | 1.0000 | 0.49     | 12.08    |  | 0.1426 | 0.1460 | 13.40    | 13.72    | 14.79 |  |
| 3 | 4  | 1.0000  | 1.0000 | 0.43     | 10.86    |  | 0.1424 | 0.1423 | 13.38    | 13.37    | 14.34 |  |
| 4 | 6  | 1.0000  | 1.0000 | 0.52     | 8.42     |  | 0.1395 | 0.1425 | 13.11    | 13.39    | 15.06 |  |
| 5 | 8  | 1.0000  | 1.0000 | 0.47     | 7.00     |  | 0.1454 | 0.1446 | 13.66    | 13.59    | 14.36 |  |
| 6 | 10 | 1.0000  | 1.0000 | 0.42     | 6.77     |  | 0.1485 | 0.1454 | 13.95    | 13.66    | 14.52 |  |

III. This straw was extracted successively with alcohol-benzene solution and hot water, loss due to extraction being 16.6%. All results calculated on original unextracted straw.

|   |    |         |        | per cent | per cent |  |        |        | per cent | per cent |       |  |
|---|----|---------|--------|----------|----------|--|--------|--------|----------|----------|-------|--|
| 1 | 1  | 1.0000‡ | 1.0000 | 0.73     | 4.83     |  | 0.1385 | 0.1408 | 11.55    | 11.74    | 15.56 |  |
| 2 | 2  | 1.0000  | 1.0000 | 0.64     | 4.99     |  | 0.1385 | 0.1410 | 11.55    | 11.76    | 15.30 |  |
| 3 | 4  | 1.0000  | 1.0000 | 0.63     | 4.57     |  | 0.1421 | 0.1428 | 11.85    | 11.91    | 15.35 |  |
| 4 | 6  | 1.0000  | 1.0000 | 0.36     | 4.43     |  | 0.1453 | 0.1449 | 12.11    | 12.08    | 15.18 |  |
| 5 | 8  | 1.0000  | 1.0000 | 0.46     | 4.06     |  | 0.1420 | 0.1426 | 11.84    | 11.89    | 15.86 |  |
| 6 | 10 | 1.0000  | 1.0000 | 0.36     | 3.82     |  | 0.1470 | 0.1460 | 12.26    | 12.17    | 14.92 |  |

\* Weight calculated on original unextracted straw—1.8248 grams.

† Weight calculated on original unextracted straw—1.0638 grams.

‡ Weight calculated on original unextracted straw—1.1990 grams.

centages of lignin and methoxyl in the subsequent experiments remained practically constant. The decrease in the percentage of lignin obtained when the reaction mixtures were refluxed for 4, 6, 8, and 10 hours was too small to be of any significance and was well within the experimental error. The percentage of methoxyl in the lignin remained practically the same throughout this series of experiments.

It has been known for many years that under certain conditions humin-like products are formed as a result of the action of strong mineral acids on sugars. Paloheimo<sup>1</sup> called attention to the fact that fructose and sucrose afford insoluble products when subjected to the action of strong mineral acids such as are used in the determination of lignin. Norman and Jenkins<sup>2</sup> showed that certain pentoses or substances yielding these sugars on hydrolysis when subjected to the action of cold 72 per cent sulfuric acid yield insoluble humin-like products. In the previous paper cited Phillips presents data showing the effect of the action of fuming hydrochloric acid on lignified plant materials that had been extracted with three different solutions. The percentage of crude lignin obtained decreased progressively with the increase in the number of extractions and the kind of extraction. This was due, no doubt, to the formation of insoluble condensation products by the action of the strong acid on certain of the carbohydrates. The experiments recorded in Parts II and III of Table 1 were conducted for the purpose of ascertaining whether these products could be removed from the lignin by prolonged hydrolysis with dilute hydrochloric acid. The general procedure followed was the same as that described in connection with the previous series of experiments. Part II shows that these carbohydrate condensation products are fully as resistant to hydrolysis as lignin itself. The percentages of "lignin" calculated on the basis of the original unextracted material are approximately 2 per cent higher than those recorded in Part I of this table, and the percentages of methoxyl in the crude lignin are correspondingly lower than those in the lignins of Part I. This is what would be expected from the nature of the impurities present in the lignin.

Part III of Table 1 shows the results of a similar series of experiments in which the wheat straw was successively extracted with alcohol-benzene solution and hot water. In none of these experiments was the percentage of lignin found affected by the length of time of heating with the dilute acid. The percentages of lignin found in the experiments recorded in Part III are of the same general order of magnitude as those shown in Part I. The purity of the lignin (seen from the data on the percentage methoxyl in the lignin) in the two series was also about the same.

In Table 2 are shown results of a series of experiments in which the only variable was the time of passage of the hydrochloric acid gas through

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<sup>1</sup> *Biochem. Z.*, **214**, 161 (1920).

<sup>2</sup> *Nature*, **131**, 729 (1933); *Biochem. J.*, **28**, 2147 (1934).



TABLE 2.—*Per cent lignin in wheat straw—effect of time of bubbling with HCl*  
(This straw was extracted successively with alcohol-benzene solution, hot water, and with 1% HCl solution, loss due to extraction being 45.2%. All results calculated on original unextracted straw.)

| EXPT. NO. | TIME ALLOWED FOR STEE BUBBLING OF FUMING HCl | WEIGHT OF SAMPLES |        | WEIGHT OF CRUDE LIGNIN |        | N IN CRUDE LIGNIN |          | ASH IN CRUDE LIGNIN |          | WEIGHT OF LIGNIN (CORRECTED FOR ASH AND CRUDE PROTEIN) |       | LIGNIN (CALCULATED ON ORIGINAL DRY MATERIAL) |       | METHOXYL IN ASH-FREE CRUDE LIGNIN |          | TEST FOR FURFURAL |
|-----------|--|-------------------|--------|------------------------|--------|-------------------|----------|---------------------|----------|--|-------|--|-------|-----------------------------------|----------|-------------------|
|           |  | (a)               | (b)    | (a)                    | (b)    | per cent          | per cent | per cent            | per cent | (a)  | (b)   | (a)  | (b)   | per cent                          | per cent |                   |
| 1         | 1  | 1.0000*           | 1.0000 | 0.2452                 | 0.2442 | 0.64              | 5.09     | 0.2229              | 0.2221   | 12.21  | 12.17 | 15.14  | 15.14 | Negative                          |          |                   |
| 2         | 2  | 1.0000            | 1.0000 | 0.2369                 | 0.2337 | 0.63              | 5.15     | 0.2154              | 0.2125   | 11.80  | 11.64 | 15.40  | 15.40 | Negative                          |          |                   |
| 3         | 4  | 1.0000            | 1.0000 | 0.2345                 | 0.2370 | 0.68              | 5.33     | 0.2121              | 0.2144   | 11.63  | 11.74 | 15.56  | 15.56 | Negative                          |          |                   |
| 4         | 6  | 1.0000            | 1.0000 | 0.2332                 | 0.2377 | 0.71              | 5.44     | 0.2102              | 0.2141   | 11.51  | 11.78 | 15.87  | 15.87 | Negative                          |          |                   |

\* Weight calculated on original unextracted straw—1.8248 grams.

TABLE 3.—*Per cent lignin in wheat straw—effect of time of reaction with fuming HCl*  
(The straw was extracted successively with alcohol-benzene solution, hot water, and 1% HCl solution, loss due to extraction being 45.2%. All results calculated on original unextracted straw.)

| EXPT. NO. | TIME OF STANDING IN STEE BOX AFTER BUBBLING OF FUMING ACID | WEIGHT OF SAMPLES |        | WEIGHT OF CRUDE LIGNIN |        | N IN CRUDE LIGNIN |          | ASH IN CRUDE LIGNIN |          | WEIGHT OF LIGNIN (CORRECTED FOR ASH AND CRUDE PROTEIN) |       | LIGNIN (CALCULATED ON ORIGINAL DRY MATERIAL) |       | METHOXYL IN ASH-FREE CRUDE LIGNIN |          | TEST FOR FURFURAL |
|-----------|--|-------------------|--------|------------------------|--------|-------------------|----------|---------------------|----------|--|-------|--|-------|-----------------------------------|----------|-------------------|
|           |  | (a)               | (b)    | (a)                    | (b)    | per cent          | per cent | per cent            | per cent | (a)  | (b)   | (a)  | (b)   | per cent                          | per cent |                   |
| 1         | 2  | 1.0000*           | 1.0000 | 0.6060                 | 0.5974 | 0.25              | 3.31     | 0.5765              | 0.5684   | 31.53  | 31.09 | 6.47   | 6.47  | Negative                          |          |                   |
| 2         | 4  | 1.0000            | 1.0000 | 0.5588                 | 0.5532 | 0.31              | 3.45     | 0.5287              | 0.5235   | 28.97  | 28.68 | 6.52   | 6.52  | Negative                          |          |                   |
| 3         | 6  | 1.0000            | 1.0000 | 0.5574                 | 0.5628 | 0.27              | 3.64     | 0.5277              | 0.5328   | 28.91  | 29.19 | 6.88   | 6.88  | Negative                          |          |                   |
| 4         | 18   | 1.0000            | 1.0000 | 0.2505                 | 0.2534 | 0.72              | 8.50     | 0.2180              | 0.2205   | 11.94  | 12.08 | 14.91  | 14.91 | Negative                          |          |                   |
| 5         | 24   | 1.0000            | 1.0000 | 0.2484                 | 0.2489 | 0.68              | 8.05     | 0.2179              | 0.2184   | 11.95  | 11.96 | 14.87  | 14.87 | Negative                          |          |                   |
| 6         | 48   | 1.0000            | 1.0000 | 0.2467                 | 0.2490 | 0.74              | 8.06     | 0.2154              | 0.2175   | 11.80  | 11.91 | 14.53  | 14.53 | Negative                          |          |                   |

\* Weight calculated on original unextracted straw—1.8248 grams.

the reaction mixture. The boiling with the dilute acid was continued for one hour, which was the optimum reaction period determined in the previous series of experiments. In all other respects the procedure described in connection with the first series of experiments was followed. The results show that when the hydrochloric acid gas was passed through the reaction mixture for only one hour, there was an incomplete removal of certain substances associated with the lignin. This is apparent from the comparatively lower percentage of methoxyl in the lignin and, as was to be expected, the percentage of "lignin" found was also higher. It also appears that the optimum time for the passage of the hydrochloric acid gas through the reaction mixture is two hours; a longer period did not appreciably affect the percentage of lignin. The several samples of lignin obtained in the various experiments of this series were distilled with 12 per cent hydrochloric acid solution, and the distillates were tested for furfural with aniline acetate paper and with phloroglucinol solution. No furfural could be detected in any of the distillates.

The results of a series of experiments in which the time of action of the fuming hydrochloric acid on the lignified plant material was varied from 2 to 48 hours are also presented (Table 3). In all experiments, the hydrochloric acid gas was passed through the reaction mixture for two hours, and hydrolysis with the boiling dilute acid was continued for one hour. The remainder of the procedure was exactly as previously described. It is shown that the time required for the hydrolysis of the cellulose and the other substances associated with the lignin by the fuming hydrochloric acid was from 18 to 24 hours. With reaction periods of 2, 4, and 6 hours, respectively, the percentages of "lignin" found were very high due to the contamination of the lignin residues with cellulose or its degradation products. A white flocculent precipitate admixed with the lignin greatly retarded the filtration. The percentages of methoxyl found in the lignin residues from Experiments 1, 2, and 3 were correspondingly low. The crude lignin precipitates obtained in the several experiments of this series were distilled with 12 per cent hydrochloric acid solution, and the distillates were tested for furfural with aniline acetate paper and with phloroglucinol solution. In all cases the tests were negative, indicating that the crude lignins were not contaminated with furfural-yielding substances.

In Table 4 results are given of a series of experiments in which the only variable was the temperature of the fuming hydrochloric acid reaction mixture. In all the experiments the time of reaction with the fuming hydrochloric acid was 24 hours, the hydrochloric acid gas was passed through the reaction mixture for two hours, and the hydrolysis with the boiling dilute acid was continued for one hour. It will be seen that the optimum temperature range for the action of the fuming hydrochloric acid was  $+8$  to  $+20^{\circ}\text{C}$ . At  $0^{\circ}\text{C}$ . the reaction is slowed down considerably, so that in the 24 hour period allowed for the reaction some of the carbohy-

TABLE 4.—*Per cent lignin in wheat straw—effect of temperature of hydrolysis with fuming HCl*

(This straw was extracted successively with alcohol-benzene solution, hot water, and 1% HCl solution, loss due to extraction being 45.2%. All results calculated on original unextracted straw.)

| EXPT.<br>NO. | TEMPERATURE<br>OF FUMING<br>HCl | WEIGHT OF SAMPLES |        | WEIGHT OF CRUDE LIGNIN |        | N IN<br>CRUDE<br>LIGNIN |      | WEIGHT OF LIGNIN<br>(CORRECTED FOR ASH AND<br>CRUDE PROTEIN) |        | LIGNIN (CALCULATED ON<br>ORIGINAL DRY MATERIAL) |       | METHOXYL<br>IN ASH-FREE<br>CRUDE<br>LIGNIN |                      |
|--------------|---------------------------------|-------------------|--------|------------------------|--------|-------------------------|------|--|--------|---|-------|--|----------------------|
|              |                                 | (a)               | (b)    | (a)                    | (b)    | (a)                     | (b)  | (a)  | (b)    | (a)   | (b)   | per cent                                   | TEST FOR<br>FURFURAL |
| °C.          |                                 |                   |        |                        |        |                         |      |  |        |   |       |  |                      |
| 1            | -1° to 0°                       | 1.0000*           | 1.0000 | 0.3747                 | 0.3889 | 0.43                    | 5.23 | 0.3450   | 0.3582 | 18.90   | 19.63 | 10.56                                      | Negative             |
| 2            | +8° to 10°                      | 1.0000            | 1.0000 | 0.2484                 | 0.2489 | 0.68                    | 8.05 | 0.2179   | 0.2184 | 11.94   | 11.96 | 14.87                                      | Negative             |
| 3            | +18° to 20°                     | 1.0000            | 1.0000 | 0.2472                 | 0.2477 | 0.68                    | 8.09 | 0.2167   | 0.2172 | 11.87   | 11.90 | 14.54                                      | Negative             |
| 4            | +25°                            | 1.0000            | 1.0000 | 0.2620                 | 0.2610 | 0.65                    | 7.59 | 0.2315   | 0.2306 | 12.68   | 12.64 | 14.32                                      | Negative             |

\* Weight calculated on original unextracted straw—1.8248 grams.

drates remain still unhydrolyzed. On the other hand, when the reaction was allowed to proceed at 25° C., the hydrolysis of the carbohydrates was again incomplete, due to the fact that in spite of all precautions there was some loss of hydrochloric acid gas, and the residual acid accordingly became weaker and less effective. Because the temperature range in the ordinary ice box, +8 to +10° C., is readily available in most chemical laboratories, this temperature was adopted for use for the hydrolysis with the fuming hydrochloric acid.

From the data given, the following method was finally formulated for the quantitative estimation of lignin:

#### LIGNIN

##### PREPARATION OF SAMPLE

The plant material is ground in a mill fine enough to pass through an 80-mesh sieve and dried at 105° C. A weighed sample (5-10 grams) is extracted for 30 hours in a Soxhlet apparatus with an alcohol-benzene solution (32 parts by weight of 95% ethanol and 68 parts by weight of benzene). The material is dried in an oven to free it from the alcohol-benzene solution, and it is then placed in a flask of suitable size. Distilled water is added in the proportion of 150 cc. of water to 1 gram of sample, and the mixture is boiled under a reflux condenser for 3 hours. The mixture is filtered while still hot, preferably through a weighed sintered-glass crucible. The extracted material is then transferred to a flask and a 1% HCl solution is added in the proportion of 150 cc. of acid solution to 1 gram of plant material, and boiled under a reflux condenser for 3 hours. The mixture is filtered while still hot through the sintered-glass crucible used in the previous operation, washed with distilled water until free of acid, dried at 105° C., and weighed. The percentage loss due to the successive extractions with alcohol-benzene solution, hot water, and 1% HCl solution is calculated.

##### DETERMINATION

Three 1-gram samples of the extracted and dried material are weighed out in a weighing bottle and placed in the three large test tubes, G, G', and G'', and 20 cc. of fuming HCl (d. 1.212-1.223 at 15° C.) is added to each tube, care being taken to wash down with this acid any particles clinging to the sides. When all the material is wetted, another portion (30 cc.) of the fuming HCl is added. About three drops of capryl alcohol are then added, the purpose being to reduce the foaming to a minimum during the subsequent passage of the HCl gas through the reaction mixture. The three large test tubes, G, G', and G'', are placed in a wooden box (L) and surrounded with crushed ice. Tubes F, F', and F'' are lubricated with a drop of glycerol, the purpose being to allow them to move freely through the holes in the rubber stoppers. Dry HCl gas from the generator is then led into the reaction mixture through the tubes F, F', and F'' (F' and F'' are shown in the top view), which reach nearly to the bottom of the tubes G, G', and G''. The flow of the gas through the reaction mixtures in G, G', and G'' is regulated by means of the stopcocks shown in the top view. The passage of the gas through the reaction mixture is continued for 2 hours. At first, a rather slow stream of gas is passed in, but during the last 15 minutes the flow is fairly rapid. At the end of the reaction period, the flow of the gas is discontinued, and the long tubes, F, F', and F'', and the outlet tubes, N, N', and N'', of the three test tubes G, G', and G'' are disconnected from O and P. The tubes F, F', and F'' are pulled up just above the surface of the reaction mixture, and are closed by means of short pieces of rubber tubing, the ends of which have

been plugged with short pieces of glass rod. The outlet tube (N) and the outlet tubes of G' and G'' are similarly closed off. The tubes containing the reaction mixture are then placed in a cold room or ice box (temperature +8° C. to +10° C.) and allowed to remain there for 24 hours. The contents of the tubes G, G', and G'' are then transferred to one liter Erlenmeyer flasks, and care is taken to remove any material adhering either on the inside or outside of the tubes F, F', and F''. The reaction mixtures are then diluted with distilled water to a volume of 500 cc., and the flasks are connected to reflux condensers and boiled for 1 hour. Three Gooch crucibles are prepared in the usual manner, dried at 105° C., and weighed. One of the weighed crucibles, A, is then ignited on a Bunsen burner, cooled in a desiccator, and reweighed. The contents of the three flasks are allowed to cool to room temperature and filtered through the weighed Gooch crucibles. The precipitates collected in the Gooch crucibles are washed with hot water, dried at 105° C., and weighed in a weighing bottle. The crude lignin in crucible A is ignited over a Bunsen flame and the weight of the ash is determined. One of the other two Gooch crucibles is placed in a Kjeldahl flask provided with a wide neck, and the percentage of nitrogen in the crude lignin is determined by the Kjeldahl-Gunning-Arnold method. If it is desired to determine the percentage methoxyl in the lignin, the precipitate from one of the flasks is collected in a dried (105° C.) sintered-glass crucible. The weight of the lignin in the sample is computed as follows: Weight of lignin equals weight of crude lignin, minus weight of ash, minus weight of crude protein ( $N \times 6.25$ ). The percentage of lignin in the original dry unextracted material is then calculated.

### SUMMARY

A study was made of the fuming hydrochloric acid method for the quantitative estimation of lignin. The optimum conditions for carrying out this determination were ascertained, and a detailed description of the revised method is given.

## STUDIES ON THE QUANTITATIVE ESTIMATION OF LIGNIN\*

### II. A COMPARISON OF THE MODIFIED FUMING HYDROCHLORIC ACID METHOD AND OTHER METHODS COMMONLY USED

By MAX PHILLIPS and M. J. GOSS

In a previous paper Phillips<sup>1</sup> presents the results of a study of the following methods for the quantitative estimation of lignin: (1) The fuming hydrochloric acid method as originally developed in this laboratory;<sup>2</sup> (2) the older U. S. Forest Products Laboratory method,<sup>3</sup> which is essentially a modification of the method of Ost and Wilkening;<sup>4</sup> (3) the modified 72 per cent sulfuric acid method of Peterson, Walde and Hixon;<sup>5</sup> and (4) the Schwalbe method.<sup>6</sup> In a later publication by Phillips,<sup>7</sup> analytical data were presented on the quantitative estimation of lignin in wheat, rye, barley and oat straw. Lignin determinations were made by the

\* Contribution from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

<sup>1</sup> *This Journal*, 17, 277 (1934).

<sup>2</sup> *Ibid.*, 15, 118 (1932).

<sup>3</sup> Mahood and Cable, *Ind. Eng. Chem.*, 14, 933 (1922); Bray, *Paper Trade J.*, 87, No. 25, 59 (1928).

<sup>4</sup> *Chem. Z.*, 34, 461 (1910).

<sup>5</sup> *Ind. Eng. Chem. Anal. Ed.*, 4, 216 (1932).

<sup>6</sup> *Papier-Fabr.*, 23, 174 (1925).

<sup>7</sup> *This Journal*, 18, 386 (1935).

original fuming hydrochloric acid method<sup>1</sup> on unextracted straws and on materials that had previously been extracted with a 1:2 alcohol-benzene solution. Quantitative estimations of lignin were also made on the same plant materials that had been successively extracted with a 1:2 alcohol-benzene solution and with hot water, and also on plant materials successively extracted with a 1:2 alcohol-benzene solution, hot water, and a 1 per cent hydrochloric acid solution. The results obtained showed that in all four samples of straws, there was a gradual decrease in the percentage of lignin, depending on the extent of the preliminary treatment of the sample. Thus, in the case of wheat straw, the percentage of lignin in the original unextracted material amounted to 16.60, but it was only 13.42, 12.56, and 11.73 in the materials extracted with alcohol-benzene solution, alcohol-benzene solution and hot water, and alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution, respectively. The analytical data obtained in connection with the other three straws showed a similar trend. This variation in the percentage of lignin in the extracted and unextracted samples was due, as indicated in the paper published by Phillips in 1935, to the fact that certain fatty or waxy substances and their degradation products and certain insoluble humin-like substances formed by the action of the strong mineral acids on certain carbohydrates are weighed together with the lignin. Paloheimo<sup>2</sup> called attention to the fact that fructose and sucrose afford insoluble products when subjected to the action of strong mineral acids such as are used in the determination of lignin. More recently Norman and Jenkins<sup>3</sup> showed that certain pentoses or substances yielding these sugars on hydrolysis, when subjected to the action of cold 72 per cent sulfuric acid, afford some insoluble material as resistant as lignin.

In this paper analytical data are presented on the quantitative estimation of lignin by four different methods and on plant materials extracted with a 1:2 alcohol-benzene solution, with a 1:2 alcohol-benzene solution followed by extraction with hot water, and finally on material extracted successively with a 1:2 alcohol-benzene solution, hot water, and a boiling 1 per cent hydrochloric acid solution. The lignin determinations were made by the following methods: (A) the improved fuming hydrochloric acid method;<sup>4</sup> (B) the modified U. S. Forest Products Laboratory method described by Ritter, Seborg and Mitchell;<sup>5</sup> (C) the 72 per cent sulfuric acid method, as modified by Peterson, Walde, and Hixon,<sup>6</sup> and (D) the Schwalbe method.<sup>7</sup> It is necessary to state in this connection, that in the case of the analyses made by the fuming hydrochloric acid method and by the modified U. S. Forest Products Laboratory method, the plant materials received only such preliminary treatments as are indicated in the

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Biochem. Z.*, 214, 161 (1929).

<sup>3</sup> *Nature*, 131, 729 (1933); *Biochem. J.*, 28, 2147 (1934).

<sup>4</sup> Goss and Phillips, *This Journal*, 19, 841 (1936).

<sup>5</sup> *Ind. Eng. Chem. Anal. Ed.*, 4, 202 (1932).

<sup>6</sup> *Loc. cit.*

<sup>7</sup> *Loc. cit.*

TABLE 1.—*Per cent lignin in wheat straw subjected to various preliminary treatments*  
(All results calculated on original unextracted straw.)

| ANALYTICAL METHOD USED  | WEIGHT OF SAMPLE (a) | WEIGHT OF CRUDE LIGNIN (b) | N IN CRUDE LIGNIN | ASH IN CRUDE LIGNIN | WEIGHT OF LIGNIN CORRECTED FOR ASH AND CRUDE PROTEIN |        | OCH <sub>3</sub> IN ASH-FREE CRUDE LIGNIN | LIGNIN (CALCULATED ON ORIGINAL DRY MATERIAL) |               |
|---|----------------------|----------------------------|-------------------|---------------------|--|--------|---|--|---------------|
|   |                      |                            |                   |                     | (a)  | (b)    |   | (a)  | (b)           |
|   | gram                 | gram                       | per cent          | per cent            | gram   | gram   | per cent                                  | per cent                                     |               |
| Extracted with Alcohol-Benzene Solution <sup>1</sup>                        |                      |                            |                   |                     |  |        |   |  |               |
| A   | 1.0000               | 1.0000                     |                   |                     | 0.1721   | 0.1678 | 0.87                                      | 9.05   | 0.1473 0.1436 |
| B   | 1.0000               | 1.0000                     |                   |                     | 0.2003   | 0.2064 | 1.00                                      | 8.38   | 0.1711 0.1763 |
| C   | 2.0000               | 2.0000                     |                   |                     | 0.4226   | 0.4275 | 1.12                                      | 7.83   | 0.3601 0.3642 |
| D   | 2.0000               | 2.0000                     |                   |                     | 0.4376   | 0.4302 | 1.23                                      | 7.57   | 0.3709 0.3647 |
| Extracted with Alcohol-Benzene Solution and Hot Water <sup>2</sup>          |                      |                            |                   |                     |  |        |   |  |               |
| A   | 1.0000               | 1.0000                     |                   |                     | 0.1669   | 0.1684 | 0.75                                      | 3.44   | 0.1534 0.1548 |
| B   | 1.0000               | 1.0000                     |                   |                     | 0.1996   | 0.2008 | 0.93                                      | 2.59   | 0.1830 0.1840 |
| C   | 2.0000               | 2.0000                     |                   |                     | 0.4143   | 0.4038 | 1.00                                      | 2.84   | 0.3770 0.3671 |
| D   | 2.0000               | 2.0000                     |                   |                     | 0.4143   | 0.4119 | 1.05                                      | 2.81   | 0.3756 0.3733 |
| Extracted with Alcohol-Benzene Solution, Hot Water, and 1% HCl <sup>3</sup> |                      |                            |                   |                     |  |        |   |  |               |
| A   | 1.0000               | 1.0000                     |                   |                     | 0.2236   | 0.2216 | 0.83                                      | 3.42   | 0.2045 0.2027 |
| B   | 1.0000               | 1.0000                     |                   |                     | 0.2398   | 0.2335 | 0.98                                      | 3.89   | 0.2159 0.2102 |
| C   | 2.0000               | 2.0000                     |                   |                     | 0.4927   | 0.4979 | 1.02                                      | 3.49   | 0.4443 0.4489 |
| D   | 2.0000               | 2.0000                     |                   |                     | 0.4861   | 0.4838 | 1.04                                      | 3.57   | 0.4373 0.4352 |

\* Figures in parentheses represent the average of the two values for the percentages of lignin found.

<sup>1</sup> Per cent extracted by alcohol-benzene solution, 6:30.

<sup>2</sup> Per cent extracted by alcohol-benzene solution and hot water, 16:6.

<sup>3</sup> Per cent extracted by alcohol-benzene solution, hot water, and 1% HCl, 45:2.

A—Fuming hydrochloric acid method.

B—Schwalbe method.

C—Modified U. S. Forest Products Laboratory method, as described by Ritter, Seborg, and Mitchell.

D—The 72% sulfuric acid method, as modified by Peterson, Walde and Hixon.

tables. In the case of the wheat straw, corrections for the nitrogen in the crude lignin residues were made.

#### EXPERIMENTAL

The materials used were wheat straw and spruce wood. The spruce wood was used because it was desired to test these methods on a highly lignified material, such as wood, and because the lignin obtained from this wood is virtually free from nitrogen (0.1 to 0.2 per cent) and therefore no correction for nitrogen need be made. The lignin obtained from such materials as hay, straw, stalks, cobs, and hulls is always contaminated with nitrogenous complexes. From the weight of crude lignin found it is necessary to deduct the weight of ash plus the weight of crude protein ( $N \times 6.25$ ). As stated by Phillips in the original paper (1932)<sup>1</sup> this procedure is based on the assumption that the nitrogenous complexes obtained along with the lignin are in the nature of a protein, as nothing definite is known of their chemical nature. In determining the percentage of lignin in substances that yield lignins contaminated with nitrogenous substances an error is necessarily introduced because of the uncertainty of the magnitude of the correction to be applied. In the determination of the percentage of lignin in spruce wood, however, there is no such error.

The samples were ground to pass an 80-mesh sieve. The various extractions were made following the procedure described by Goss and Phillips.<sup>2</sup> The percentage methoxyl in the lignin was determined by the Kirpal and Bühn<sup>3</sup> modification of the Zeisel method.<sup>4</sup> All percentages of lignin were calculated on the basis of the original dry unextracted material.

The results of the lignin determinations made on wheat straw are given in Table 1, and those made on spruce wood are given in Table 2. The percentages of lignin determined in wheat straw and in spruce wood show a progressive decrease, depending on the extent of the preliminary treatment of the sample. Thus in the case of wheat straw analyzed by the fuming hydrochloric acid method, the percentage of lignin in the sample extracted with an alcohol-benzene solution was 13.67 (mean of two values), but it was only 12.85 and 11.15 in the samples extracted with an alcohol-benzene solution and hot water, and alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution, respectively. In the case of spruce wood, the percentages (mean values) were 26.94, 25.52, and 24.29, respectively. The results obtained by the three other analytical methods show the same trend. However, the results obtained by the fuming hydrochloric acid method show less variation than those obtained by the other three methods. This was particularly true in the case of the lignin values found for the three samples of wheat straw. Thus the differences of the mean values for the percentages of

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *This Journal*, 19, 349 (1936).

<sup>3</sup> *Ber.*, 47, 1084 (1914); *Monatsh.*, 36, 853 (1915).

<sup>4</sup> *Monatsh.*, 6, 989 (1885); 7, 406 (1886).



TABLE 2.—*Per cent lignin in spruce wood subjected to various preliminary treatments*  
(All results calculated on original unextracted wood.)

| ANALYTICAL METHOD USED  | WEIGHT OF SAMPLE |        | WEIGHT OF CRUDE LIGNIN |        | ASH IN CRUDE LIGNIN |        | WEIGHT OF LIGNIN CORRECTED FOR ASH |       | OCH <sub>3</sub> IN ASH-FREE CRUDE LIGNIN |          | LIGNIN (CALCULATED ON ORIGINAL DRY MATERIAL) |     |
|---|------------------|--------|------------------------|--------|---------------------|--------|------------------------------------|-------|---|----------|--|-----|
|   | (a)              | (b)    | (a)                    | (b)    | (a)                 | (b)    | (a)                                | (b)   | per cent                                  | per cent | (a)  | (b) |
| gram  |                  |        |                        |        |                     |        |                                    |       |   |          |  |     |
| Extracted with Alcohol-Benzene Solution <sup>1</sup>                        |                  |        |                        |        |                     |        |                                    |       |   |          |  |     |
| A   | 1.0000           | 1.0000 | 0.2899                 | 0.2883 | 0.41                | 0.2887 | 0.2871                             | 15.46 | 27.02                                     | (26.94)* | 26.87  |     |
| B   | 1.0000           | 1.0000 | 0.3053                 | 0.3041 | 0.81                | 0.3028 | 0.3016                             | 15.20 | 28.34                                     | (28.28)  | 28.23  |     |
| C   | 2.0000           | 2.0000 | 0.6152                 | 0.6144 | 0.48                | 0.6123 | 0.6115                             | 15.14 | 28.65                                     | (28.63)  | 28.61  |     |
| D   | 2.0000           | 2.0000 | 0.6174                 | 0.6163 | 0.69                | 0.6132 | 0.6121                             | 15.18 | 28.69                                     | (28.66)  | 28.64  |     |
| Extracted with Alcohol-Benzene Solution and with Hot Water <sup>2</sup>     |                  |        |                        |        |                     |        |                                    |       |   |          |  |     |
| A   | 1.0000           | 1.0000 | 0.2972                 | 0.2982 | 0.57                | 0.2956 | 0.2965                             | 15.10 | 25.48                                     | (25.52)  | 25.56  |     |
| B   | 1.0000           | 1.0000 | 0.3089                 | 0.3082 | 0.46                | 0.3075 | 0.3068                             | 15.02 | 26.50                                     | (26.47)  | 26.44  |     |
| C   | 2.0000           | 2.0000 | 0.6264                 | 0.6264 | 0.46                | 0.6235 | 0.6235                             | 15.09 | 26.87                                     | (26.87)  | 26.87  |     |
| D   | 2.0000           | 2.0000 | 0.6239                 | 0.6244 | 0.64                | 0.6199 | 0.6204                             | 15.13 | 26.71                                     | (26.72)  | 26.74  |     |
| Extracted with Alcohol-Benzene Solution, Hot Water, and 1% HCl <sup>3</sup> |                  |        |                        |        |                     |        |                                    |       |   |          |  |     |
| A   | 1.0000           | 1.0000 | 0.3669                 | 0.3688 | 0.67                | 0.3645 | 0.3663                             | 15.43 | 24.24                                     | (24.29)  | 24.35  |     |
| B   | 1.0000           | 1.0000 | 0.3863                 | 0.3875 | 0.64                | 0.3839 | 0.3850                             | 15.53 | 25.53                                     | (25.56)  | 25.60  |     |
| C   | 2.0000           | 2.0000 | 0.7717                 | 0.7716 | 0.57                | 0.7673 | 0.7673                             | 15.40 | 25.51                                     | (25.51)  | 25.51  |     |
| D   | 1.0000           | 1.0000 | 0.3892                 | 0.3842 | 0.56                | 0.3871 | 0.3821                             | 15.50 | 25.74                                     | (25.70)  | 25.67  |     |

\* Figures in parentheses represent the average of the two values for the percentages of lignin found.

<sup>1</sup> Per cent extracted by alcohol-benzene solution, 6.4.

<sup>2</sup> Per cent extracted by alcohol-benzene solution and hot water, 13.8.

<sup>3</sup> Per cent extracted by alcohol-benzene solution, hot water, and 1% HCl, 33.5.

A—Ritting method.

B—Schwabe method.

C—Modified U. S. Forest Products Laboratory method, as described by Ritter, Seborg, and Mitchell.

D—The 72% sulfuric acid method, as modified by Peterson, Walde and Hixon.

lignin found in wheat straw extracted by the three different methods indicated in the table were 0.82 and 1.70 (differences between 13.67 and 12.85 and between 12.85 and 11.15). The corresponding differences in the lignin percentages found by the three other methods were: Schwalbe method, 1.02 and 3.63; Forest Products Laboratory method, 1.50 and 3.28; Peterson, Walde, and Hixon method, 1.67 and 3.66.

In the case of the three samples of spruce wood analyzed by the four different methods the corresponding differences in the percentages were: Fuming hydrochloric acid method, 1.42 and 1.23; Schwalbe method, 1.81 and 0.91; Forest Products Laboratory method, 1.76 and 1.36; Peterson, Walde, and Hixon method, 1.94 and 1.02. The greater variation in the percentages of lignin found in the three samples of wheat straw analyzed by the four different methods can be attributed to the fact that this plant material contains a greater percentage of hot water and 1 per cent hydrochloric acid extractives (10.6 and 28.6 per cent, respectively), than does spruce wood (7.4 and 19.7 per cent, respectively). As already mentioned, certain carbohydrates when treated with strong mineral acids such as are used in the determination of lignin afford insoluble products as resistant to hydrolysis as lignin itself and these contaminating substances are therefore weighed as lignin. The hot water extractives and the 1 per cent hydrochloric acid extractives undoubtedly contain these carbohydrates in substantial amounts. As shown by the results, fuming hydrochloric acid produces a smaller percentage of these interfering substances than does 72 per cent sulfuric acid or the mixture of 72 per cent sulfuric acid and hydrochloric acid used in the other methods. However, even when the fuming hydrochloric acid method is used, these interfering substances must be removed previously, as otherwise quite erroneous lignin values will be obtained. This is particularly true in the case of the other three methods.

Attention is called to the progressive increase in the percentages of methoxyl found in the lignins obtained from the three samples of wheat straw analyzed by the four different methods. The samples that had been extracted only with an alcohol-benzene solution yielded lignins containing smaller percentages of methoxyl than those samples which had been successively extracted with an alcohol-benzene solution and hot water, and with an alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution. This is exactly what would be expected, for as the lignin becomes purer and less contaminated with substances free from methoxyl or with substances containing a much smaller percentage of methoxyl than lignin itself, the percentage of methoxyl found in the lignin residue must necessarily show an increase. In general, it may be stated that the percentages of methoxyl found in the lignins isolated from the three samples of straw by the fuming hydrochloric acid and Schwalbe methods were greater than those obtained by the other two methods.

Attention is called to the fact that the percentages of lignin found by

the fuming hydrochloric acid method in the two samples of wheat straw extracted only with an alcohol-benzene solution and with an alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid were 13.67 and 11.15, respectively, a difference of 2.52 per cent. In the case of the same samples analyzed by the three other methods the percentages were: Schwalbe method, 16.32 and 11.67, a difference of 4.65 per cent; Forest Products Laboratory method, 17.01 and 12.23, a difference of 4.78; the Peterson, Walde, and Hixon modification of the 72 per cent sulfuric acid method, 17.28 and 11.95, a difference of 5.33 per cent. In the case of the samples of spruce wood analyzed by the four methods the differences were not so great but the trend was the same.

### SUMMARY

1. A comparison was made between the percentages of lignin found in three different samples of wheat straw and spruce wood that had received the following preliminary treatments: (1) Extracted only with an alcohol-benzene solution; (2) extracted successively with an alcohol-benzene solution and hot water; (3) extracted successively with an alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution. The lignin determinations were made by the modified fuming hydrochloric acid method, the Schwalbe method, the modified U. S. Forest Products Laboratory method, and the Peterson, Walde and Hixon modification of the 72 per cent sulfuric acid method. The results show that the percentages of lignin determined by all four methods in wheat straw and in spruce wood decreased in proportion to the extent of the preliminary treatment of the sample.

2. The difference between the percentages of lignin in the sample extracted only with an alcohol-benzene solution and the one extracted successively with an alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid solution was the greatest when the determinations were made by the Peterson, Walde, and Hixon modification of the 72 per cent sulfuric acid method. The differences between the percentages of lignin found in the same samples by the other three methods tested decreased in the following order: (1) Modified U. S. Forest Products Laboratory method; (2) Schwalbe method; (3) modified fuming hydrochloric acid method.

3. The results show that unless the plant material is first successively extracted with an alcohol-benzene solution, hot water, and 1 per cent hydrochloric acid before being subjected to the action of the strong mineral acids, erroneous lignin values are obtained.

4. The percentage of lignin found by the modified fuming hydrochloric acid method is in all probability a closer approximation to the true lignin content of the plant material than is that found by the other three methods studied.

## BOOK REVIEWS

**Chronica Botanica**, Vol. 1. By FR. VERDOORN, Editor-in-Chief. This first volume, distributed last fall, announces the ambitious program of presenting each year "a review of the important Current Research in all branches of Plant Science." With this is included a large body of information regarding botanists and botanical institutions, together with a "calendar of botanical events."

On the whole, the result is fairly successful, although institutions are very unevenly represented, depending on the industry or modesty of their correspondents. Biographical sketches and photographs of the following recently deceased well-known American botanists are included: Prof. James Barkley Pollock, 1863-1934; Dr. E. Bartholomew, 1852-1934; Th. H. Macbride, 1848-1934; Dr. N. L. Britton, 1859-1934; Mrs. E. G. Britton, 1858-1934; Prof. R. T. Fisher, 1876-1934; Prof. F. L. Stevens, 1871-1934; Dr. Karl F. Kellerman, 1879-1934; and Homer C. Skeels, 1873-1934.—NEIL E. STEVENS.

**Organic Chemistry**. By HOWARD J. LUCAS, Associate Professor of Organic Chemistry, California Institute of Technology. VI+686 pp., 41 figs. 14×22.5 cm. American Book Co., New York, 1935. Price \$3.60.

This book can best be described by a quotation from the preface. "A satisfactory textbook of organic chemistry not only should present the subject matter in a systematic and logical manner, but also should correlate it with principles. In the attempt to do these things in this text, the author has adopted certain procedures, as follows: Emphasis has been placed upon class reactions, rather than upon the reactions of individual compounds; relationships with inorganic compounds are made use of, when possible; energy relationships are discussed and their significance pointed out; the underlying principles of molecular structure are early presented and applied throughout to individual cases; the electrochemical nature of radicals is indicated and their effect upon properties noted; and numerous applications to the electronic theory of valence are made. . . . Important aspects of the electron theory are the type of valence bond, that is, whether it is an ionic, a covalent, a coordinate covalent, or a hydrogen bond; the possibility of resonance; and the influence of radicals upon electronic configurations. All of these factors are important, and their application to the problems of structure and properties will continue, as they have done in the past, to lead to a better understanding of organic chemistry."

The author has accomplished his purpose very well indeed and has produced a rather unusual textbook on organic chemistry. Physico-chemical principles are introduced early in the book and are applied in nearly every chapter.

The first three chapters are devoted to a discussion of general principles, such as valence, atomic and molecular structures, and chemical constitution. Chapters 4 to 24, inclusive, are devoted to a discussion of the aliphatic and alicyclic hydrocarbons and to their derivatives and substitution products. In Chapters 25 to 37 consideration is given to the chemistry of the aromatic series of compounds. The remaining chapters are devoted to a consideration of the more complicated compounds, such as aliphatic hydroxy, aldehyde and keto acids, amino acids, proteins, carbohydrates, terpenes, heterocyclic compounds, alkaloids, drugs, and synthetic dyes.

The reviewer has found the information in the book up to date and has failed to discover any serious error. However, he wishes to call attention to the fact that the name Crafts is misspelled whenever reference is made to the reaction of Friedel and

Crafts (pp. 341, 349, 429, 458 and 665). In a modern textbook on organic chemistry it is rather surprising to note that the old Theory of Types is utilized in connection with the consideration of the structure of ethers, esters and acid anhydrides. The advisability of treating such diverse classes of organic compounds as ethers, esters, anhydrides and acid chlorides as a group is also questioned. These minor commissions, however, in no way detract from the value of the book as a whole. The reviewer considers this one of the very best books of its class and without any hesitation recommends it to the teacher and student of elementary organic chemistry as well as to the advanced student of this subject.—MAX PHILLIPS.

## TUESDAY—MORNING SESSION

### REPORT ON PLANTS

By O. B. WINTER (Michigan Agricultural Experiment Station,  
East Lansing, Mich.), *Referee*

During the past year the Referee and the Associate Referees on Plants have worked on methods for making the following determinations on plants and plant materials: iron, potassium, sodium, copper, lead, chlorine, iodine, fluorine, carbohydrates, and forms of nitrogen. The greater part of these studies was done by associates, most of whom will report the progress made on their particular problems. Some of this work resulted in slight modifications of present methods and deletion of methods that are now obsolete owing to the recent advances made in analytical chemistry. For example, one method given in this chapter for the determination of calcium requires that the solution be made very slightly acid, then 10 cc. of 0.5 *N* HCl, 10 cc. of 2.5 per cent  $\text{H}_2\text{C}_2\text{O}_4$ , 15 cc. of saturated  $(\text{NH}_4)_2\text{C}_2\text{O}_4$ , and finally 8 cc. of  $\text{NaC}_2\text{H}_3\text{O}_2$  be added to precipitate the  $\text{CaC}_2\text{O}_4$ . Now it is known that those reagents bring the solution to approximately pH 5, at which point  $\text{CaC}_2\text{O}_4$  is precipitated and interfering elements remain in solution. The present-day analyst adds a drop of methyl red and makes the solution neutral to that indicator. As this newer method is given in the chapter on plants, the old method should be deleted.

#### IRON

The sulfocyanate colorimetric method given for the determination of iron is satisfactory and accurate. However, most analysts prefer a titration method if it is convenient, rapid, and accurate. That iron can be determined by titrating  $\text{FeCl}_3$  with  $\text{TiCl}_3$ <sup>1</sup> has been known for a long time. The method, however, is used very little. Possibly this is because  $\text{TiCl}_3$  oxidizes very easily and changes quite rapidly. However, with certain precautions the method has been used in some laboratories and found to be rapid; it also appears to be accurate. The procedure is as follows:

After preparing the sample as for the colorimetric method, take a convenient aliquot of the solution and oxidize the iron by adding dropwise a very dilute solution of  $\text{KMnO}_4$  until a very, very faint permanganate color persists. Add 5 cc. of 10%  $\text{NH}_4\text{CNS}$  and titrate with approximately 0.02 *N*  $\text{TiCl}_3$  to the disappearance of the red color. Approximately a 0.02 *N* solution of  $\text{TiCl}_3$  should be prepared, standardized with a known iron solution, and kept in the dark in a well-filled container. It must be standardized against the iron solution each time it is used or every few hours when making a large number of determinations. This is easily and quickly done if a standard iron solution is kept on hand.

Table 1 gives the results of iron determinations made by two analysts by the A.O.A.C. colorimetric method and also by the titanous chloride

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<sup>1</sup> Sutton, *Volumetric Analysis*, 11th Ed., p. 240 (1924).

titration method on a synthetic solution, on a few plant materials, and on dried milk. The data appear to be promising, and the method deserves further study.

TABLE 1.—*Comparison of a colorimetric and a titration method for the determination of iron*

| SAMPLE             | Fe      |                  |           |
|--------------------|---------|------------------|-----------|
|                    | PRESENT | FOUND BY BUTLER* |           |
|                    |         | COLORIMETRIC     | TITRATION |
|                    | mg.     |                  | per cent  |
| Synthetic solution | 0.558   |                  | 0.559     |
|                    |         |                  | per cent  |
| Pablum (baby food) |         |                  | 0.028     |
|                    |         |                  | 0.029     |
| Whole wheat        |         |                  | 0.0047    |
| Grass              |         |                  | 0.015     |
|                    |         |                  | 0.013     |
| Leaves             |         |                  | 0.017     |
|                    |         |                  | 0.017     |
| Dried milk         |         | 0.0021 %         | 0.0014    |

\* Assistant in Chemistry, Michigan Agr. Exp. Sta., East Lansing, Mich.

† Analyst for Mead Johnson and Co., Evansville, Ind.

## COPPER

Miss Lillian Butler of this laboratory made a number of copper determinations by a modification of the diethyldithiocarbamate method proposed by Haddock and Evers.<sup>1</sup> The method is based on the fact that sodium diethyldithiocarbamate reacts with copper to give the copper salt of diethyldithiocarbamic acid. This salt has a golden-brown color and can be extracted quantitatively from an aqueous solution with carbon tetrachloride. In very dilute solutions the color is directly proportional to the amount of copper present. Otherwise it is necessary to draw a curve, using known amounts of copper. The following is the method as modified:

### MODIFIED HADDOCK-EVERS METHOD

#### REAGENT

*Standard copper solution.*—0.377 gram of dehydrated copper sulfate dissolved in 500 cc. of water. 100 cc. diluted to 1 liter. 1 cc. = 0.03 mg. of Cu.

#### PROCEDURE

Burn 1–5 grams of the material in a muffle at dull redness (about 500°C.). Add 2 cc. of HNO<sub>3</sub> and about 10 cc. of water and heat to boiling. If only a small amount of insoluble residue remains, transfer directly to a 150 cc. separatory funnel. If much insoluble residue remains, filter through washed asbestos with suction and wash with 2% HNO<sub>3</sub>. (Do not use ordinary filter paper because of danger of copper contaminations.) Transfer to the separatory funnel. Add a piece of litmus paper

<sup>1</sup> *Analyst*, 57, 495 (1932).

and then add in the following order and shake after each addition, 10 cc. of 10% citric acid, ammonia until slightly alkaline (not above pH 9), 10 cc. of 0.1% diethyldithiocarbamate reagent, and 10 cc. of carbon tetrachloride. Shake vigorously for several minutes, allow the layers to separate, and dry the stem of the funnel with a cotton applicator. Draw off the carbon tetrachloride solution into a dry test tube. Compare in a colorimeter with a standard copper solution prepared in a similar manner. Read the amount of copper from a curve previously prepared with known amounts of copper. It is advisable to run blanks, but if all the apparatus is washed carefully before use with dilute  $\text{HNO}_3$ , the blank will be negligible.

Table 2 gives the results of copper determinations made by three collaborators on three synthetic solutions and on a few samples of plant materials. These results are in fair agreement, and only in a few exceptional cases so far apart that evidently they should not be included.

TABLE 2.—Copper determinations

|   |         | BUTLER   | DEMILLER* | CONN†                      |
|---|---------|--|-----------|----------------------------|
|   | mg./cc. | mg./cc.  | mg./cc.   | mg./cc.                    |
| Synthetic Soln No. 1<br>( $\text{CuSO}_4$ )   | 0.034   | 0.034<br>0.035<br>0.034                        | 0.033     | 0.0372<br>0.0351           |
| Synthetic Soln No. 2<br>(.002% Fe, .16% $\text{Ca}_3(\text{PO}_4)_2$ ,<br>.25% $\text{NH}_4\text{Cl}$ , and 1% $\text{HCl}$ ) | 0.0037  | 0.0039<br>0.0038<br>0.0042<br>0.0039           | 0.0032    | 0.0040<br>0.0048<br>0.0046 |
| Synthetic Soln No. 3<br>(.001% Fe and 5% $\text{HNO}_3$ )   | 0.0037  | 0.0037<br>0.0037<br>0.0037                     |           | 0.0092<br>0.113            |
|   |         | per cent                                       | per cent  | per cent                   |
| Whole wheat   |         | 0.0045<br>0.0041<br>0.0048                     | 0.0040    | 0.0031<br>0.0032           |
| Grass No. 11  |         | 0.0011<br>0.0012<br>0.0012<br>0.0010<br>0.0010 | 0.0021    | 0.0011                     |
| Leaves No. 12   |         | 0.0098<br>0.0100<br>0.0096<br>0.0100           | 0.0110    |                            |
| Pablum (baby food)  |         | 0.0012<br>0.0013                               | 0.0013    |                            |

\* These results were obtained by the method used by Mead Johnson and Co.

† Sealtest System Laboratories, Inc., 120 Broadway, New York. Results were obtained by the method used in the Research Laboratory at Eutaw Place, Baltimore, Maryland.



## SODIUM AND POTASSIUM

The indirect method for the determination of sodium in plants is not entirely satisfactory. Since no direct method is given in the 1930 edition of *Methods of Analysis* for the determination of this element, the referee made a careful study of the work that has been done since the last revision. The associate referee had made no formal report since 1933. However, because of the results given in that report<sup>1</sup> and the results of some work that has been done (unpublished) on the problem since that report was made, the referee is of the opinion that the procedure given should be made tentative and then studied further. The method was published in *This Journal*, 19, 71 (1936).

No mention is made of the perchloric acid method for determining sodium and potassium anywhere in the 1930 Edition of *Methods of Analysis*. The associate referee gave a short report on this method in 1933. This procedure is a standard method<sup>2</sup> used by a number of analysts and is applicable to plant materials. Hence this should be made a tentative method. The procedure was published in *This Journal*, 19, 71 (1936).

## FLUORINE

The work of this laboratory on fluorine has been directed along two lines: 1st, trying to get a suitable fixative for burning the sample which will keep the fluorine in such a form that it may be completely volatilized when the ash is distilled with perchloric acid according to the Willard and Winter method;<sup>3</sup> and 2nd, trying to determine the fluorine in the distillate colorimetrically by a modification of the Smith and Dutcher method.<sup>4</sup> The fading of zirconium-alizarine lake is a measure of the amount of fluorine present. This method was chosen because the color fading takes place in the presence of an excess of acid, which generally exists in the distillate from plant ash. It should be emphasized here that when there is a considerable amount of salt present in the neutralized distillate and thorium nitrate is being used for titrating, the end point is not sufficiently sharp to determine accurately the small amount of fluorine present in many plant materials.

Of the several fixatives tried for holding the fluorine during the ignition of the sample, magnesium acetate proved most satisfactory, and with it fluorine added to most plant materials could be recovered quantitatively. However, only about 95–97 per cent recovery was obtained from certain grasses that are high in silicates.

## FLUORINE

*Colorimetric Method*

*Preparation of sample and separation of fluorine.*—Place 5–25 grams of material in a crucible, add sufficient 5% magnesium acetate solution to moisten completely

<sup>1</sup> Butler, *This Journal*, 17, 275 (1934).

<sup>2</sup> Smith and Ross, *J. Am. Chem. Soc.*, 47, 1020 (1925).

<sup>3</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 7 (1933).

<sup>4</sup> *Ibid.*, 6, 61 (1934).

(but no more), dry in an oven for at least 24 hours, and ash in a muffle at dull redness (about 500°C.). Brush the ash into a 75–100 cc. distillation flask whose delivery tube has an upward curve to prevent bumping over. Wash the crucible several times with water and a small amount of perchloric acid, adding the wash solution to the flask. Connect the apparatus for distillation according to the Willard and Winter method.<sup>1</sup> Remove the stopper, add perchloric acid slowly from a pipet until effervescence ceases, and then add approximately 15 cc. more. Replace the stopper, boil at 135–140°C., and collect the distillate in a 100 cc. volumetric flask. After this flask is filled (Distillate 1), collect another 50 cc. (Distillate 2) to be certain that all the fluorine has been volatilized.

*Preparation of zirconium-alizarine lake*—Dissolve 0.87 gram of  $\text{Zr}(\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$  in 100 cc. of  $\text{H}_2\text{O}$ , and 0.17 gram of sodium alizarinate in 100 grams of  $\text{H}_2\text{O}$ . Mix equal parts of the two solutions and dilute the mixture (1–4) with  $\text{H}_2\text{O}$ .

*Colorimetric procedure.*—Make up a series of standards in Nessler tubes or test tubes of about 80 cc. capacity by placing 0.02, 0.04, 0.06, 0.09, 1.20 mg. of fluorine in each tube, respectively. Add water to make about 50 cc., 10 cc. of approximately 1–1 HCl, mix thoroughly, add 2 cc. of the dye solution, and bring all to the same level with water. Again mix thoroughly, place the tubes in a steam bath for 30 minutes, and cool. For unknowns take aliquots whose fluorine contents fall within the above range of standards. The fluorine is determined from the nearest standard.

If quantities of fluorine below the range given are to be determined, less dye should be used; if quantities above, more dye should be used.

When a series of standards is prepared as directed and placed in a tintometer, the individual tubes show a fading in color as the amount of fluorine increases. Hence the fluorine content of an unknown sample can easily be determined with a fair degree of accuracy from the nearest standard.

The data in Table 3 show the results of some colorimetric fluorine determinations on synthetic solutions, an alfalfa, a grass, and on the alfalfa and the grass after fluorine had been added.

The results (Table 3) indicate that fairly accurate fluorine determinations can be made by the colorimetric method on fluoride solutions when no interfering elements are present, either by making the determinations directly or after distillation with perchloric acid. In determining the fluorine in such materials as alfalfa or a grass the duplicate determinations agree fairly well, but the fluorine content decreases with the size of the sample. This undoubtedly is due to incomplete distillation of the fluorine and not to the colorimetric method.

#### LEAD

For the determination of lead in plants, work was done in the referee's laboratory with a modification of the Fischer-Leopoldi method<sup>2</sup> formulated by Winter, Robinson, Lamb, and Miller. The method is short, convenient, and easily manipulated, and it is believed by the authors to be accurate. This method should be compared with other methods and studied collaboratively.

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 7, 265 (1935).

TABLE 3.—*Colorimetric determination of fluorine*

| SAMPLE  | FLUORINE |                                  | F.P.M.       |
|---|----------|----------------------------------|--------------|
|   | ADDED    | FOUND                            |              |
|   | mg.      | mg.                              |              |
| NaF solution                                  | 0.076    | 0.076<br>0.068<br>0.076<br>0.084 |              |
| NaF solution distilled with HClO <sub>4</sub> | 0.076    | 0.080<br>0.084<br>0.076          |              |
| 5 grams of alfalfa                            |          | 0.050<br>0.053                   | 10.0<br>10.1 |
| 10 grams of alfalfa                           |          | 0.099<br>0.091                   | 9.9<br>9.1   |
| 20 grams of alfalfa                           |          | 0.167                            | 8.4          |
| 5 grams of grass                              |          | 0.053                            | 10.6         |
| 10 grams of grass                             |          | 0.099                            | 9.9          |
| 5 grams of alfalfa                            | 0.076    | 0.122                            |              |
| 5 grams of grass                              | 0.076    | 0.114                            |              |
| Blank on reagents                             |          | 0.000<br>0.008                   |              |

RECOMMENDATIONS<sup>1</sup>

It is recommended that the following changes be made in the Chapter on Plants for the 1935 revision of *Methods of Analysis*:

(1) Secs. 1, p. 102 "Directions for Sampling"; 6, p. 103, "Iron and Aluminum"; and 7, p. 103, "Micro Method for Iron Only," be made official, first action.

(2) Secs. 10, p. 105, "Micro Method for Calcium"; and 29, p. 110, "Method II, Micro Method for Phosphorus," be made official, final action.

(3) Sec. 9, p. 104, part 1, "Official Method for Calcium," be deleted.

(4) Sec. 13, p. 106, "Magnesium," be revised (see *This Journal*, 19, 71 (1936)).

(5) That the titanous chloride titration method for the determination of iron given in this report be studied further collaboratively.

(6) That the studies on different forms of nitrogen be continued.

(7) That the method for the determination of fluorine given in this report be compared with other methods and studied collaboratively.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 80 (1936).

(8) That the method for the determination of lead referred to in this report be compared with other methods and studied collaboratively.

(9) That the perchloric acid method for sodium and potassium given in this report be made tentative and studied collaboratively.

(10) That the magnesium uranyl acetate method for sodium given in this report be made tentative and studied collaboratively.

(11) That the reports of the associate referees be adopted.

## REPORT ON LESS COMMON METALS IN PLANTS

By J. S. MCHARGUE (Kentucky Agricultural Experiment Station, Lexington, Kentucky), *Associate Referee*

During the past year further work was done on methods for the determination of iodine in plant material, and a paper on the iodine content of some forage crops and foods was published.<sup>1</sup>

A sample of plant material for A.O.A.C. collaborative work was sent out to two laboratories that had consented to make iodine determinations according to any procedure they might choose.

The following results were received:

| <i>Iodine in Plant Material</i>     |                   |     |                 |
|-------------------------------------|-------------------|-----|-----------------|
| <i>Collaborator</i>                 |                   |     | <i>per cent</i> |
| Halvorson, H. A.<br>St. Paul, Minn. |                   | a — | .0030           |
|                                     |                   | b — | .0043           |
|                                     |                   | c — | .0050           |
|                                     |                   | d — | .0038           |
|                                     |                   | Av. | .0040           |
| Young, D. W.<br>Lexington, Ky.      | Fusion Method     | a — | .0030           |
|                                     | " "               | b — | .0039           |
|                                     | " "               | c — | .0040           |
|                                     |                   | Av. | .0036           |
|                                     |                   |     |                 |
| Young, D. W.                        | Combustion Method | a — | .0038           |
|                                     | " "               | b — | .0040           |
|                                     |                   | Av. | .0039           |
| McHargue, J. S. Fusion Method       |                   |     | — .0044         |

The data show that iodine can be determined reasonably accurately by fusion with alkali on plant material containing a relatively large quantity of the element and also by the combustion method on plant material containing small amounts of the element.

<sup>1</sup> McHargue, Young and Calfee, *J. Am. Soc. Agron.*, 27, No. 7 (1935).

It is recommended<sup>1</sup> that the fusion method for the determination of iodine in plant material containing relatively large quantities of the element, and the combustion method reported last year, for plant material low in iodine, be adopted as tentative methods and that further collaborative work be done.

## REPORT ON TOTAL CHLORINE IN PLANTS

By HERBERT L. WILKINS (Bureau of Plant Industry, Division of Forage Crops and Diseases, National Agricultural Research Center, Beltsville, Md.), *Associate Referee*

On the basis of further work, the associate referee revised the method given in last year's report<sup>2</sup> and recommended that it be adopted as a tentative method and studied further with respect to suitable materials and to details of the preparation and use of the aqueous iodine solution. The method has been published,<sup>3</sup> consequently only the work that forms the basis for the changes that have been made since the last report will be presented.

In 1934 it was noted that there was an apparent discrepancy between the results obtained with large and small aliquots of a silver solution. In searching for the reason for this, many titrations were made in which freshly, or differently prepared reagents were used in various sequences and proportions. It was observed that very slow titrations gave higher results than did more rapid ones. This led to the discovery that there was an appreciable amount of chlorine in the starch solution. When the starch was washed repeatedly with cold water as directed under reagent (d) it gave a practically chloride-free solution.

It was also evident from this series of titrations that the successive batches of the biiodate indicator reagent must be very carefully adjusted. That correct results could be obtained from various aliquots by close adjustment of the indicator and washing the chlorides out of the starch was demonstrated by the analysis of four 5.01 ml. and three 24.99 ml. portions of standard solution of hydrochloric acid containing on an average 13.93 mg. and 69.38 mg., respectively (Table 3). Now  $24.99/5.01 \times 13.93 = 69.48$  mg., or 0.10 mg. more than the result obtained by direct titration. Conversely,  $5.01/24.99 \times 69.38 = 13.91$ , or 0.02 mg. less than the result obtained by direct titration. In both cases the difference between the calculated and the observed value is 0.14 per cent of the calculated value. There is thus seen to be as good agreement in results obtained on the large and small aliquots as there is between the analyses and the nominal value of the standard solution.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 50 (1936).

<sup>2</sup> *This Journal*, 18, 379-82 (1935).

<sup>3</sup> *Ibid.*, 19, 72 (1936).

As exact adjustment is difficult with the indicator solution a way to avoid its use was sought. It had been observed<sup>1</sup> that the amount of potassium iodide required to give the initial end point with biiodate was proportional to the amount of biiodate present. The records made in preparing the indicator showed that the amounts of biiodate and iodide involved conformed to those required by the equation,  $\text{KH}(\text{IO}_3)_2 + 10\text{KI} + 11\text{HNO}_3 \rightarrow 11\text{KNO}_3 + 6\text{I}_2 + 6\text{H}_2\text{O}$ . It seemed likely, therefore, that the biiodate served mainly as a convenient source of free iodine, which if true, could just as well be furnished directly as a solution of iodine. Several such solutions were tried with varying success. The solution of iodine in dilute sulfuric acid designated as reagent (f) proved to be very satisfactory. It is easily prepared from iodine crystals, needs no adjustment before use, and gives good end points.

TABLE 1.—*Collaborative results on sample of grass*

| ASSOCIATE REFEREE |                 | WINTER        |                 |                 |
|-------------------|-----------------|---------------|-----------------|-----------------|
| SAMPLE WEIGHT     | PROPOSED METHOD | SAMPLE WEIGHT | PROPOSED METHOD | VOLHARD METHOD  |
| <i>grams</i>      | <i>per cent</i> | <i>grams</i>  | <i>per cent</i> | <i>per cent</i> |
| 2                 | 0.626           | 2             | 0.631           |                 |
| 2                 | 0.626           | 2             | 0.632           |                 |
| 2                 | 0.626           | 2             | 0.630           |                 |
| 2                 | 0.627           | 1             | 0.635           |                 |
| 2                 | 0.627           | 1             | 0.640           |                 |
| 2                 | 0.624           | ?             |                 | 0.630           |
| 2                 | 0.626           | ?             |                 | 0.613           |
| 2                 | 0.622           |               |                 |                 |
| 2                 | 0.625           |               |                 |                 |
| 2                 | 0.625           |               |                 |                 |
| Blank             | 0.00            | Blank         | 0.00            | ?               |
| Average           | 0.625           |               | 0.634           | 0.622           |
| Average deviation | $\pm 0.001$     |               | $\pm 0.003$     |                 |

This is conclusive evidence that the production of free iodine was the sole function of the biiodate and that the essential end point reaction is not the production of free iodine (since this may be present in quantity from the first) but the addition of iodide ions to the system. Without these the starch-iodide color does not develop. This fact, which was stated in Treadwell and Hall<sup>2</sup> as early as 1905, is easily overlooked because *ordinarily* of the three necessary components—starch, iodine, and iodide ions—*iodine* is the one usually employed in the limiting sense.

Most of the potassium permanganate was added near the end of the

<sup>1</sup> *This Journal*, 17, 268 (1934); 18, 379 (1935).

<sup>2</sup> *Analytical Chemistry*, Vol. I, p. 267 (1905). John Wiley and Sons, New York.

nitric acid digestion to destroy oxalic acid,<sup>1</sup> which results from the action of nitric acid on the sample.

Stainless steel wire cloth of 30-mesh, woven from number 27 (B & S gage) wire containing 18 per cent chromium and 8 per cent nickel, has been found to be a very satisfactory material for the disks for use in the funnel under the filter paper.

The precision of the method, one of its most obvious characteristics, is illustrated by the titrations obtained by C. O. Miller, Bureau of Animal Industry, Beltsville, Md., on ten successive equal aliquots of a solution of silver nitrate. They were 9.57, 9.59, 9.58, 9.58, 9.57, 9.52, 9.57, 9.55,

TABLE 2.—*Results on samples from Winter*  
NaCl solution (1 ml. contains 5.000 mg. of chlorine)

| ASSOCIATE REFEREE |                 |          | WINTER        |                 |          |                |
|-------------------|-----------------|----------|---------------|-----------------|----------|----------------|
| SAMPLE WEIGHT     | PROPOSED METHOD |          | SAMPLE WEIGHT | PROPOSED METHOD |          | VOLHARD METHOD |
| mg.               | mg./ml.         | per cent | mg.           | mg./ml.         | per cent | per cent       |
| 50.05             | 4.969           | 99.38    | 50.00         | 4.975           | 99.50    |                |
| 50.05             | 4.961           | 99.22    | 50.00         | 4.970           | 99.40    |                |
| 50.05             | 4.964           | 99.28    | 50.00         |                 |          |                |
| 50.05             | 4.969           | 99.38    |               |                 |          |                |
| Averages          | 4.966           | 99.32    |               | 4.973           | 99.45    |                |
| Dried sugar beets |                 |          |               |                 |          |                |
| grams             | ml. KI          | per cent | grams         | ml. KI          | per cent | per cent       |
| 2                 | 1.10            | 0.059    | 2             | 1.10            | 0.055    |                |
| 2                 | 1.08            | 0.058    | 2             | 1.10            | 0.055    |                |
| 2                 | 1.11            | 0.059    | 2             | 1.15            | 0.057    |                |
| 2                 | 1.14            | 0.060    | 4             | 2.35            | 0.058    |                |
| 2                 |                 |          | 4             | 2.40            | 0.060    |                |
|                   |                 |          | ?             |                 |          | 0.063          |
|                   |                 |          | ?             |                 |          | 0.063          |
| Averages          |                 | 0.059    |               |                 | 0.057    | 0.063          |

9.58, and 9.56 ml., with an average of 9.567 ml., and an average deviation from this mean of  $\pm 0.014$  ml. or  $\pm 0.015$  mg. of chlorine. When the entire process was involved and both Miller and the associate referee did part of the work on each of ten successive determinations on the collaborative sample the results were 11.76, 11.76, 11.77, 11.78, 11.79, 11.73, 11.77, 11.70, 11.74, and 11.75 ml., with an average of 11.755, and an average deviation of  $\pm 0.020$  ml. or  $\pm 0.021$  mg. of chlorine, which in this case was  $\pm 0.001$  per cent. In Tables 1 and 2 are given the results on 12 titrations

<sup>1</sup> *This Journal*, 18, 379 (1935).

obtained by O. B. Winter, Michigan Agricultural Experiment Station, East Lansing, Mich. The average difference between the replicates and their respective averages is  $\pm 0.024$  ml. or  $\pm 0.024$  mg. of chlorine.

The collaborative results on the same grass sample and on other samples sent to the associate referee are given in Tables 1 and 2. Besides the ten determinations on the grass sample, the results of various other determinations by Winter are given. The averages obtained in the two laboratories differ by only 0.009 per cent, with 0.622 and 0.640 the low and high values, respectively. Table 2 shows results on the Winter samples, which he analyzed by both his usual method and that of the associate referee. The latter made four determinations on the solution, averaging 4.966 mg./ml., or 99.32 per cent of 5.000 mg./ml. Winter's two results averaged 4.973 mg./ml., or 99.45 per cent. The two analysts differ from

TABLE 3.—*Results obtained by the associate referee on different aliquots of a standard solution of hydrochloric acid by the proposed method*

| SAMPLE WEIGHT | CHLORINE FOUND | DEVIATION FROM<br>AMOUNT TAKEN | RECOVERED |
|---------------|----------------|--------------------------------|-----------|
| mg.           | mg.            | mg.                            | per cent  |
| 13.92         | 13.95          | +0.03                          | 100.23    |
| 13.92         | 13.88          | -0.04                          | 99.73     |
| 13.92         | 13.93          | +0.01                          | 100.09    |
| 13.92         | 13.95          | +0.03                          | 100.23    |
| Average       | 13.93          | $\pm 0.03$                     | 100.07    |
| 69.42         | 69.36          | -0.06                          | 99.91     |
| 69.42         | 69.37          | -0.05                          | 99.92     |
| 69.42         | 69.41          | -0.01                          | 99.98     |
| Average       | 69.38          | $\pm 0.04$                     | 99.94     |
| Grand average |                | $\pm 0.03$                     | 100.01    |

each other by about seven parts in 5000 and from the nominal value of 5.000 mg./ml. by about three parts in 500. On the sample of beets one analyst reports 0.057 per cent and the other 0.059. Winter used both large and small samples and obtained slightly higher results with the small sample of grass, but the small sample of beets gave the lower results, so there is no apparent effect of size of sample.

The accuracy of the method is indicated by two sets of data. One deals with the agreement between the quantities found and those taken for analysis; the other concerns the agreement between the results obtained on the same sample by the proposed and by other methods in the hands of the same or different analysts. Table 3 gives the results obtained by the associate referee, using the method on standard hydrochloric acid pre-



pared from carefully distilled constant-boiling acid. Two grams of C.P. sucrose was added to each sample. The amounts found range from 99.73 to 100.23, and average 100.01 per cent of the quantities taken. The average difference between the amount taken and that found is  $\pm 0.03$  mg., a value quite similar to that found for successive replicates. Using the proposed method on the sodium chloride solution, the associate referee reports an average of 99.32 per cent and Winter an average of 99.45 per cent of the amount taken (Table 2). The average of all determinations on known samples obtained by both analysts when using the proposed method is 99.71 per cent. In addition, Winter determined the chlorine in both the grass and the beets by his usual method, obtaining an average of 0.063 per cent on the beet and 0.622 per cent on the grass samples. These figures compare very favorably with his 0.057 and 0.634 per cent and with the associate referee's 0.059 and 0.625 per cent, respectively, obtained by the proposed method. Several samples were obtained from E. M. Bailey, of the Connecticut Agricultural Experiment Station, New Haven, Conn., on which his laboratory determined the water-soluble

TABLE 4.—*Results obtained on samples furnished by E. M. Bailey*

| MATERIAL   | SAMPLE NUMBERS | ASSOCIATE REFEREE | BAILEY'S LABORATORY* |
|------------|----------------|-------------------|----------------------|
|            |                | per cent          | per cent             |
|            |                | NaCl              |                      |
| Feed       | 8233           | 1.49              | 1.41 and 1.50        |
| Feed       | 8671           | 4.77              | 4.45                 |
| Feed       | 8912           | 1.42              | 1.43                 |
| Feed       | 9057           | 0.77              | 0.91                 |
|            |                | Cl <sub>2</sub>   |                      |
| Fertilizer | 8630           | 13.6, 13.1, 11.8  | 10.95                |

\* All results are on the water-soluble material.

chlorides by the "usual volumetric method." The silver chloride was filtered off before the back titration of the excess silver was made, except on Sample 8630. Samples 8671 and 8630 were rather coarse and non-uniform in appearance. On the assumption that they were analyzed in that condition, the associate referee mixed them in the unopened bottles and weighed out the samples as received. As may be seen in Table 4 the agreement of the two laboratories is excellent on Samples 8233 and 8912; not so good as desired on Samples 8671 and 9057, and very unsatisfactory on Sample 8630, which was a fertilizer. All the other samples were feeds, probably mostly of plant origin.

If the results obtained on all of the samples are considered, the accuracy of the method is quite satisfactory and the precision is all that could be expected from ordinary volumetric procedures. With a moderate

amount of experience the analyst should be able to consider that his results were within 0.05 mg. of the amount present, and this to a large extent is independent of the amount taken.

As both the associate referee and Winter found the blank determinations to be very near zero, there would seem to be no need to make them after the reagents have been checked.

## REPORT ON CARBOHYDRATES IN PLANTS

By J. T. SULLIVAN (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

Last year<sup>1</sup> the permanganate method for the estimation of reduced copper formed during the determination of reducing sugars in plants was discussed. Some causes of poor results by the method as it has been adopted tentatively, *Methods of Analysis*, A.O.A.C., 1930, 113, 43 (a) and (b), were pointed out and improvements were indicated. Further experience of the associate referee during the past year shows that the procedure, which is described in detail in *This Journal*, 19, 72 (1936), is superior in accuracy and uniformity of results to the present tentative procedures.

The procedure recommended does not involve any fundamental changes in the procedures already adopted. The changes may be summarized as solution of the  $\text{Cu}_2\text{O}$  in the acid before dilution with water, greater emphasis on care to dissolve all the copper particles, and the use of an indicator to give a sharper end point. It is recommended that the procedure indicated replace the procedures already adopted. Collaborative work on the modified method will be attempted.

The associate referee is also engaged upon studies for the determination of sucrose and starch.

### RECOMMENDATIONS<sup>2</sup>

It is recommended—

(1) That the description of the tentative methods for the determination of reduced copper by the volumetric permanganate method appearing in *Methods of Analysis*, A.O.A.C., 1930, 113, 43 (a) and (b) be replaced by the procedure suggested.

(2) That the determination of sucrose in plants be further studied.

(3) That studies be made upon the determination of starch in plants.

No report on forms of nitrogen was given by the associate referee.

No report on sodium and potassium was given by the associate referee.

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<sup>1</sup> *This Journal*, 18, 382 (1935).

<sup>2</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 51, 72 (1936).

## REPORT ON LIGNIN

By MAX PHILLIPS (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.), *Referee*

In 1932 the quantitative estimation of lignin by the use of fuming hydrochloric acid was described, *This Journal*, 15, 118. Further work on the method was continued during the past year. The results obtained are given in detail in a paper published in *This Journal*, 19, 341 (1936). Briefly, the following facts were ascertained:

(1) The plant material should be extracted successively with 1:2 alcohol-benzene solution, boiling water, and boiling 1 per cent hydrochloric acid before being subjected to the action of the fuming hydrochloric acid.

(2) The optimal temperature for hydrolysis of the plant material with fuming hydrochloric acid is  $+8$  to  $+10^{\circ}$  C.

(3) The hydrolysis with the fuming hydrochloric acid should be continued for 24 hours.

(4) The passage of the dry hydrogen chloride through the reaction mixture should be continued for 2 hours.

(5) The optimal time for the hydrolysis with the dilute acid is 1 hour.

It is recommended<sup>1</sup> that the modified fuming hydrochloric acid method be adopted by the Association as a tentative method for the quantitative estimation of lignin.

REPORT ON ENZYMES<sup>2</sup>

By A. K. BALLS, *Referee*, and W. S. HALE (U. S. Bureau of Chemistry and Soils, Washington, D. C.)

The continuation of the work on the proteinase of flour<sup>3</sup> confirms the previously reported findings that this enzyme is activated by glutathione and by cysteine, and therefore belongs undoubtedly to the class of papain-like ferments that is so widespread in the plant world. The effect of an excessive amount of a proteolytic enzyme in bread dough is an unfavorable one, and results in the formation of an unduly soft and sticky product.

The determination of proteinase activity in flour may serve therefore as a guide in estimating the tendency to flow that the dough will exhibit. This property, however, may be conveniently measured directly by a simple method that is believed to be new.

A paste of equal weights of carbon dioxide-charged water and flour saturated with CO<sub>2</sub> while dry softens when kept at 30° in a constant

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 51 (1936).

<sup>2</sup> Food Research Division Contribution No. 300.

<sup>3</sup> Balls and Hale, *This Journal*, 18, 135 (1935).

temperature bath. The degree of fluidity of the paste is easily measured by dropping a lead shot onto the paste, and noting the time it takes for it to sink below the surface. It is interesting to note, as shown in the table, that the fluidity of the paste increases very rapidly in the presence of a trace of cysteine hydrochloride, as might be anticipated if the causative agent is a protein-digesting enzyme. The paste of unbleached flour softens gradually. The same flour after bleaching makes a paste that hardly softens at all. The addition of a small amount of cysteine causes both pastes to soften rapidly. The slow increase in the fluidity of the paste made from flour bleached with an oxidizing agent (chlorine) indicates that the oxidation of the activator of the flour proteinase is responsible for the tougher doughs that usually result from the use of bleached flours.

TABLE 1.—*Showing method of determining rate of softening of a flour paste*  
(Whole wheat flour; 5 g. flour + 5 cc. CO<sub>2</sub>-charged H<sub>2</sub>O; temp. 30°; BB shots  
dropped on surface of paste from a height of 5 in.)

| AGE OF PASTE<br>WHEN SHOT WAS DROPPED— | TIME REQUIRED FOR IMMERSION OF SHOT—     |   |
|--|--|---|
|  | UNBLEACHED FLOUR                         | CL-BLEACHED FLOUR                         |
| min.                                   | min.                                     | min.                                      |
| 0                                      | 3.0                                      | not immersed in 7 hours                   |
| 30                                     | 1.5                                      | not immersed in 7 hours                   |
| 120                                    | 1.0                                      |   |
|  | UNBLEACHED FLOUR<br>+ 5 MG. CYSTEINE—HCl | CL-BLEACHED FLOUR<br>+ 5 MG. CYSTEINE—HCl |
| 0                                      | 1.60                                     | 3.0                                       |
| 30                                     | 0.50                                     | 0.30                                      |
| 60                                     | 0.25                                     | 0.10                                      |

## REPORT ON PAPAIN

By T. L. SWENSON (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.), *Associate Referee*

In reviewing the results of the collaborators the associate referee was impressed by their agreement, for in measuring proteinase action the allowable limit of error between two operators is usually large. The same operator should check his results much more closely than this, however. Two of the five collaborators (A and D) had had considerable experience with this method, and their results check absolutely. The other three collaborators were trying this method, presumably, for the first time. Yet the results obtained by two of these three agree consistently with the results of the experienced men. The third operator (E) seems to have had trouble with the buffer solution, and it is quite evident that his solutions were not at the same pH as the others. This is a very interesting point,

inasmuch as E obtained much larger protein-splittings than any of the others. It may mean that the peptidases in the preparations have more alkaline pH optima than the proteinase, or that the pH optimum of the proteinase on casein is not accurately described. The latter explanation is doubted, but it may be worth while to investigate it.

All the activated samples, as one collaborator points out, reacted differently to hydrogen sulfide. This is ordinarily explained by assuming different ratios of active to inactive enzyme in the original preparations. If the cost were not prohibitive, it would be well to use glutathione as an activator, for there is less chance of enzyme destruction by it.

These papain preparations were not particularly good, being too old, but they were the best obtainable at the time. Papain usually loses its activity in about a year. The inactive enzyme is also destroyed, so that it is necessary to activate an old preparation, and it may be so far gone that no amount of reduction can bring back the enzymic property.

The associate referee expresses sincere thanks and appreciation to the collaborators and invites them to continue this or similar work.

It is recommended<sup>1</sup> that the method discussed be studied further, preferably on more active preparations, including preparations of bromelin, and that further information regarding the best method of activating the enzyme be sought.

The results obtained in this collaborative work are summarized in Tables 1 and 2.

TABLE 1.—*Papain activity expressed as titrable differences with 0.1 N alcoholic KOH*

|        |   | I    | I+H <sub>2</sub> S | II   | II+H <sub>2</sub> S | III  | III+H <sub>2</sub> S |
|--------|---|------|--------------------|------|---------------------|------|----------------------|
|        |   | cc.  | cc.                | cc.  | cc.                 | cc.  | cc.                  |
| 5 mg.  | A | 0    | 0                  | 0.25 | 0.75                | 0.15 | 0.75                 |
|        | B | 0.05 | 0.10               | 0.20 | 0.55                | 0.30 | 0.55                 |
|        | C | 0    | 0.22               | 0.25 | 0.40                | 0.05 | 0.55                 |
|        | D | 0    | 0                  | 0.25 | 0.75                | 0.15 | 0.75                 |
|        | E | 0.60 | 0.80               | 1.00 | 1.30                | 0.80 | 1.00                 |
| 10 mg. | A | 0    | 0.05               | 0.50 | 1.05                | 0.20 | 1.15                 |
|        | B | 0    | -0.05              | 0.10 | 0.95                | 0.10 | 0.75                 |
|        | C | 0    | 0.40               | 0.45 | 1.00                | 0.10 | 0.90                 |
|        | D | 0    | 0.05               | 0.50 | 1.05                | 0.20 | 1.15                 |
|        | E | 0.90 | 1.00               | 1.80 | 2.70                | 1.50 | 1.70                 |
| 25 mg. | A | 0    | 0                  | 0.85 | 1.60                | 0.50 | 1.50                 |
|        | B | 0    | 0.10               | 0.25 | 1.15                | 0.35 | 1.05                 |
|        | C | 0    | 0.60               | 0.65 | 1.30                | 0.70 | 0.98                 |
|        | D | 0    | 0                  | 0.85 | 1.60                | 0.50 | 1.50                 |
|        | E | 1.90 | 2.20               | 3.50 | 3.90                | 3.10 | 3.40                 |

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 51 (1936).

TABLE 2.—Value of—or units per mg. Calculated for 5 mg. and 10 mg. doses

| PREPARATION | COLLABORATOR | DOSE (NOT ACTIVATED) |        |      | DOSE (ACTIVATED BY H <sub>2</sub> S) |        |      |
|-------------|--------------|----------------------|--------|------|--------------------------------------|--------|------|
|             |              | 5 mg.                | 10 mg. | Av.  | 5 mg.                                | 10 mg. | Av.  |
| I           | A            | 0                    | 0      | 0    | 0                                    | 0      | 0    |
|             | B            | 0.01                 | 0      | 0    | 0.02                                 | 0.01   | 0.02 |
|             | C            | 0                    | 0      | 0    | 0.04                                 | 0.04   | 0.04 |
|             | D            | 0                    | 0      | 0    | 0                                    | 0.01   | 0.01 |
|             | E            | 0.12                 | 0.09   | 0.11 | 0.16                                 | 0.10   | 0.13 |
| II          | A            | 0.05                 | 0.05   | 0.05 | 0.15                                 | 0.11   | 0.13 |
|             | B            | 0.04                 | 0.01   | 0.03 | 0.11                                 | 0.10   | 0.11 |
|             | C            | 0.05                 | 0.05   | 0.05 | 0.08                                 | 0.10   | 0.09 |
|             | D            | 0.05                 | 0.05   | 0.05 | 0.15                                 | 0.11   | 0.13 |
|             | E            | 0.20                 | 0.18   | 0.19 | 0.26                                 | 0.27   | 0.27 |
| III         | A            | 0.03                 | 0.02   | 0.03 | 0.15                                 | 0.12   | 0.14 |
|             | B            | 0.06                 | 0.01   | 0.04 | 0.11                                 | 0.08   | 0.10 |
|             | C            | 0.01                 | 0.01   | 0.01 | 0.11                                 | 0.09   | 0.10 |
|             | D            | 0.03                 | 0.02   | 0.03 | 0.15                                 | 0.12   | 0.14 |
|             | E            | 0.16                 | 0.15   | 0.16 | 0.20                                 | 0.17   | 0.19 |

No report on paper and paper material was given by the referee. The Executive Committee decided that no methods for these materials and for fibers would be included in the fourth edition of *Methods of Analysis*.

## REPORT ON WATERS, BRINE AND SALT

By C. H. BADGER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The determination of fluorine in drinking water is of importance from a public health standpoint, since it has been shown that small quantities of this element have caused mottled enamel of the teeth. The method for its determination presented for adoption is based upon the experience and work of Wichmann and Dahle of the Food and Drug Administration, and the referee is deeply indebted to them for help and advice in the formulation of the method, which was published in *This Journal*, 19, 99 (1936). Collaborative results on a sample of water by this method were reported by the Referee on Fluorine in Foods in 1933, *This Journal*, 17, 204 (1934).

### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the method for the determination of fluorine in water suggested by the referee be adopted as tentative.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 51 (1936).

(2) That the method for the determination of arsenic (*Methods of Analysis*, A.O.A.C., 1930, p. 420, 78 and 79), using the Marsh-Berzelius apparatus, be dropped. This method has become obsolete through the use of the Gutzeit method.

## REPORT ON MINERAL SALTS AND EFFERVESCENT SALTS

By A. E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The following methods for effervescent and non-effervescent salts are presented.

### 1. EFFERVESCENT SALTS

Unless otherwise directed express results as g./100 g.

#### (a) PREPARATION OF SAMPLE—TENTATIVE

Avoid extreme temperatures and humidities when opening and storing samples. If the sample is coarser than 20-mesh, grind so that all will pass through a 20-mesh sieve, but avoid undue grinding so that as much material as possible will be retained on a 60-mesh sieve, and transfer without delay to glass-covered jars of suitable size (do not fill jars over  $\frac{3}{4}$  full). Keep the closed jars in a cool place. Before opening a jar for the removal of sample for analysis, alternately invert and roll it, 25 or more times if necessary, in order to obtain a homogeneous mixture. Weigh out all needed portions as nearly at the same time as possible.

#### (b) MOISTURE—TENTATIVE

Place about 0.5 gram sample in a dry counterpoised weighing bottle (50 mm. high and 25 mm. in diameter fitted with a ground-glass stopper). Weigh the stoppered bottle containing the sample and record the weight of the sample. Place the bottle in an aluminum holder at about a 20° angle. (The holder is preferably made from a piece of sheet aluminum 2×3½ inches. To form the long sides bend up at right angles a strip about  $\frac{3}{8}$  inches wide. Make two cuts about  $\frac{3}{8}$  inches long from the corners of one end, so that a portion of the metal may be bent up to form an angle in which the bottom end of the weighing bottle may rest. About one inch from the other end cut out a flap, about  $\frac{3}{8}$  inches wide and 1 inch long, to support the top end of the weighing bottle at the proper angle.) Remove the bottle top and place it on the front end of the holder. Now place the holder containing the bottle with sample and top in a modified Abderhalden drier.<sup>1</sup> Carefully turn on the vacuum and allow to run for 5 minutes, then turn on the heat. Keep the temperature of the inner tube of the drier at 100° for 30 minutes. Discontinue the heat, and turn off the vacuum. Remove the vacuum tube and replace with a CaCl<sub>2</sub> drying tube. Now allow dried air to flow into the drier until the ground-glass joint is removable. Remove the weighing bottle from the drier and quickly place the tops on the bottles. Place the bottles in a desiccator and allow to cool. Weigh, and repeat the drying until constant weights are obtained. Record the loss in weight.

<sup>1</sup> E. P. Clark, *Ind. Eng. Chem.*, 20, 306 (1928).

## 2. NON-EFFERVESCENT SALTS

These salts usually consist of sodium sulfate or magnesium sulfate or a mixture of these salts.

### (a) PREPARATION OF SAMPLE—TENTATIVE

If the sample appears wet or damp, place it in a cool, dry place until crystals are formed. Then place upon a clean, cool, dry surface, and quarter. Place such an amount of the quartered sample as is required for analysis in a thin layer upon a large watch-glass or a sheet of glass. Stir, and mix the sample at 15 minute intervals until all the crystals disintegrate when touched with a stirring rod.

Transfer to a suitable sized jar (do not fill jar over  $\frac{3}{4}$  full), and proceed as directed under 1(a), beginning with "before opening a jar."

### (b) MATTERS INSOLUBLE IN WATER—TENTATIVE

Put 10 grams of the sample in a 400 cc. beaker, cautiously add 200 cc. of  $H_2O$  at room temperature, and proceed as directed under **XX XVII**, 120.<sup>1</sup>

### (c) MATTERS INSOLUBLE IN ACID—TENTATIVE

Put 10 grams of the sample in a 400 cc. beaker, cautiously add 200 cc. of HCl (1+19), then proceed as directed under **XXXVII**, 121.<sup>1</sup>

### (d), (e), (f) SULFATE, CALCIUM, AND MAGNESIUM

Proceed as directed under **XXXVII**, 122, 123, 124, 125.<sup>1</sup>

It is recommended that these methods be further studied.

## REPORT ON DAIRY PRODUCTS

By GUY G. FRARY (State Chemical Laboratory, Vermillion,  
S. Dak.), *Referee*

### CHEESE

It is recommended<sup>2</sup> that the methods for the determination of ash and total chlorides in cheese recommended by the associate referee, *This Journal*, 18, 401 (1935), be adopted tentatively.

This recommendation is made for the following reasons: The present method for ash and salt, *Methods of Analysis*, A.O.A.C., 1930, 239, 93, was recommended to be dropped, first action in 1934. If final action is taken this year, as recommended, the 1935 edition of *Methods* will be without a method for this determination. The work of the associate referee on the proposed methods, while not as extensive as desirable, is believed to be sufficient to justify adoption of the methods as tentative.

### LACTOSE IN MILK

It is recommended that the instructions for preparation of the precipitating reagent for proteins in the determination of lactose in milk,

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930.

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 60 (1936).



*Methods of Analysis*, A.O.A.C., 1930, 216, 12(b), be corrected so as to read "200 cc. of glacial acetic acid" instead of "20 cc. of glacial acetic acid." The error in the present instructions was cited in 1933, and since that time more study has been given the matter, hence the change should be made without further delay. It has been shown, *This Journal*, 17, 345, that the original publication of the formula called for the larger volume of acetic acid.

It is also recommended that a slight change in section 13, *Methods of Analysis*, A.O.A.C., 1930, 216, be made in the second paragraph so that it will read as follows: "Add 1 cc. of the acid  $\text{Hg}(\text{NO}_3)_2$  solution or 30 cc. of the  $\text{HgI}_2$  solution (an excess of these reagents does no harm), fill to the mark, shake frequently for at least 15 minutes, filter through a dry filter, and polarize." The change recommended here is simply definite instructions for thorough agitation, it having been found that clarification is not complete and filtration is difficult without adequate shaking. It is recommended also that further study in the use of the precipitant,  $\text{Hg}(\text{NO}_3)_2$ , be made.

#### GELATIN IN DAIRY PRODUCTS

It is recommended that to the qualitative test for gelatin, p. 223, 26, be added a clarifying note to describe the precipitates which may be expected and thereby render more certain correct interpretation of results; also a note giving instructions for preparation of the sample in case of cottage cheese.

#### CREAM

It is recommended that the method for the determination of the amount of added water in cream, p. 225, 32, be made official, final action. First action was taken on this matter in 1926.

#### DRIED MILK

It is recommended that the method for the determination of fat in dried milk, *This Journal*, 15, 75 (1932), be made official, final action. First action was taken last year. As (a) and (b) represent alternative procedures the method should so indicate. It is recommended that the lactic acid method, *This Journal*, 16, 436 (1933), adopted as tentative in 1934, be dropped.

#### SEDIMENT IN MILK

Because of the widespread use in control laboratories of means of detecting visible dirt and sediment in milk, it is desirable that a standard method for such detection be included in *Methods of Analysis*. Laboratory workers using the chemical methods for milk examination use also the physical method for detection of sediment in milk. The American Public Health Association has adopted a method for this examination, which has

been found practicable and probably as accurate as the determination involved will permit. It is, therefore, recommended that this Association adopt the method for sediment test described in Standard Methods of Milk Analysis, 1934, 6th ed., pages 44-46, as tentative. Collaborative study of the method need not be carried out, since the American Public Health Association has given it thorough study, for which it should be given credit.

#### EXTRANEOUS MATTER IN BUTTER

An associate referee was appointed after last year's meeting, but no report will be presented. It is recommended that study on a method for determining extraneous matter in butter and in other dairy products be continued.

#### ICE CREAM

It now appears that none of the numerous modifications of the Babcock fat test is applicable to a determination of fat in ice cream in the hands of different operators. It is therefore recommended that study of such modification be discontinued.

#### MILK PROTEINS

Further study on the determination of milk proteins is recommended.

#### CITRIC ACID IN MILK

Hartmann and Hillig published in 1932, *This Journal*, 15, 643, the description of a method for determination of citric acid in milk, which gave encouraging results. A year later the same workers, *Ibid.*, 16, 427, published a paper in which they showed that by means of a citric acid determination there might be reached an approximation of the amount of milk solids present in a manufactured milk product. Further study indicates that this determination is of sufficient value to justify its adoption as a tentative method of analysis. Therefore, it is recommended that without further collaborative study the method proposed by Hartmann and Hillig for the determination of citric acid in milk and milk products be adopted as tentative.

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No report on butter was given by the associate referee.

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#### REPORT ON CHEESE

By C. B. STONE (U. S. Food and Drug Administration,  
Cincinnati, Ohio), *Associate Referee*

The collaborative work on the proposed methods for the determination of ash and total chloride could not be finished because authentic samples were not secured.

It is recommended<sup>1</sup>—

(1) That the official method for the determination of fat in cheese (*Method of Analysis, A.O.A.C.*, 1930, 239, 97) be dropped (final action).

(2) That the official method for the determination of moisture in cheese, Method I (*Ibid.*, 238, 91), be amended by inserting after the word "cheese" in the second line the words "and process cheese" (final action).

(3) That the methods proposed by the associate referee for the determination of ash and total chlorides in cheese be studied collaboratively.

(4) That the official method for the determination of ash and salt in cheese (*Ibid.*, 239, 93) be dropped, final action to be taken when the collaborative work is completed on the proposed method for ash and total chlorides in cheese.

## REPORT ON MALTED MILK

By FRED HILLIG (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Last year the Association recommended that the method proposed for the determination of fat in malted milk be studied collaboratively and that further work be done on the determination of the Reichert-Meissl value on fat so extracted. In the report to the Association in 1933, *This Journal*, 17, 350 (1934), results obtained by the proposed method and the tentative method on the determination of fat in 5 samples of malted milk were given. They showed that when applied to malted milk the tentative and the proposed methods give concordant results in the hands of an individual analyst. It was noted, however, that with the exception of one sample the average results by the tentative method were slightly higher than those by the proposed method. This may be due to the incomplete removal of non-fat material. The average non-fat material obtained by the tentative method for 11 determinations was 1.3 mg. and the range was from 0.4 mg. to 2.7 mg.

Later, the method was modified and was incorporated in a paper, entitled "Unified Method for the Determination of Fat in Foods with Special Reference to the Evaluation of their Butterfat Content," *This Journal*, 18, 455 (1935). Results are given on the determination of fat by the proposed and official methods on a variety of food products, as well as Reichert-Meissl values on fat extracted by the proposed method.

This year a chocolate-flavored malted milk was prepared in the laboratory and submitted to collaborators for the determination of fat and R-M value by the proposed procedure. The results are given in Table 1.

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 60 (1936).

TABLE 1.—*Collaborative results*

| COLLABORATOR | FAT             | R M VALUE |
|--------------|-----------------|-----------|
|              | <i>per cent</i> |           |
|              | 7.23            | 17.38     |
| 1            | 7.23            | 17.33     |
|              | 7.18            |           |
| 2            | 7.15            | 17.04     |
|              | 7.10            |           |
| 3            | 7.17            | 17.30     |

It will be seen that in the hands of different analysts closely agreeing duplicates were obtained, also that the results of the three collaborators checked very well.

In 1932 two samples of malted milk were sent to 11 collaborators for the determination of fat by the tentative method. The results were not entirely satisfactory and were not given in tabular form in the associate referee's report, *This Journal*, 15, 528 (1932). It is believed advisable at this time, however, to submit these results in order to compare them with the results given in Table 1 obtained by the proposed method.

It will be noted that in the hands of individual analysts closely agreeing duplicates were usually obtained. However, when the determinations of the various analysts are considered, the results are not satisfactory. This may be due to (1) the small quantity of sample taken, (2) the occurrence of emulsions, and (3) difficulty in removal of non-fat material from the dried fat. In the proposed method provisions have been made to eliminate these difficulties. Ten grams of material may be conveniently used as a sample. It is subjected to acid hydrolysis, treated with filter cel, chilled, filtered, dried to a fine powder, and extracted with a single solvent, petroleum ether. The dried fat is free of non-fat material, making resolution with petroleum ether unnecessary.

No work was done on the recommendations pertaining to the determination of casein and to the type of mounting for the microscopical identification, as suggested by Ballard.<sup>1</sup>

It is recommended<sup>2</sup>—

(1) That the determination of fat and R-M value by the method proposed be further studied collaboratively with a view to its adoption as tentative.

(2) That last year's recommendation for the determination of casein be carried over.

(3) That last year's recommendation for the study of the type of mounting suggested by Ballard be carried over.

<sup>1</sup> *This Journal*, 17, 351 (1934).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 60 (1936).

TABLE 2.—*Collaborative results on fat obtained in 1932*

| COLLABORATOR | SAMPLE NO. 1    |      | SAMPLE NO. 2    |      |
|--------------|-----------------|------|-----------------|------|
|              | <i>per cent</i> |      | <i>per cent</i> |      |
| 1            | 9.36            |      | 8.28            |      |
|              | 9.30            |      | 8.29            |      |
|              | 9.27            | 9.31 | 8.30            | 8.29 |
| 2            | 8.90            |      | 8.08            |      |
|              | 9.00            | 8.95 | 8.09            | 8.09 |
| 3            | 9.37            |      | 8.30            |      |
|              | 9.38            | 9.38 | 8.27            | 8.29 |
| 4            | 9.37            |      | 8.61            |      |
|              | 9.37            | 9.37 | 8.61            | 8.61 |
| 5            | 9.31            |      | 8.50            |      |
|              | 9.29            | 9.30 | 8.42            | 8.44 |
|              |                 |      | 8.41            |      |
| 6            | 9.68            |      | 8.70            |      |
|              | 9.76            | 9.72 | 8.67            | 8.69 |
| 7            | 9.35            |      | 8.46            |      |
|              | 9.42            | 9.39 | 8.36            | 8.41 |
| 8            | 9.17            |      | 8.31            |      |
|              | 9.19            | 9.18 | 8.25            | 8.28 |
| 9            | 9.20            |      | 8.18            |      |
|              | 9.27            | 9.17 | 8.16            | 8.17 |
|              | 9.05            |      |                 |      |
| 10           | 9.08            |      | 7.97            |      |
|              | 9.03            | 9.06 | 8.04            | 8.01 |
| 11           | 8.90            | 8.90 | 8.03            | 8.03 |
| Max.         |                 | 9.72 |                 | 8.69 |
| Min.         |                 | 8.90 |                 | 8.01 |

## REPORT ON DRIED MILK

By FRED HILLIG (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The method for the determination of lactic acid that was recommended for adoption last year<sup>1</sup> was found to be unsatisfactory, and con-

<sup>1</sup> *This Journal*, 18, 402 (1935).

sequently a revised method was submitted for collaborative study. However, the collaborative results were so unsatisfactory that the data are not recorded here.

Recently a colorimetric method appeared in the literature;<sup>1</sup> it is based on a color reaction with ferric chloride. This color principle was adapted to the determination of lactic acid in milk products. No time was afforded for collaborative work, but from the promising results obtained by the associate referee it is believed that the procedure will develop into a serviceable and accurate method for the determination of lactic acid in milk. Because of the lack of collaborative work, however, it would appear to be undesirable to publish the present method for the determination of lactic acid in the forthcoming revision of *Methods of Analysis*.

It is recommended<sup>2</sup>—

- (1) That the calcium oxalate method recommended last year as tentative be dropped.
- (2) That the colorimetric method be further studied with a view to its adoption as tentative.
- (3) That methods for the detection of neutralizers be studied.

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No report on ice cream was given by the associate referee.

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## REPORT ON MILK PROTEINS

By WILLIAM E. PETERSEN (Division of Dairy Husbandry,  
University of Minnesota, Minneapolis, Minn.),  
*Associate Referee*

### CASEIN

It was recommended last year<sup>3</sup> that further work be done on the present tentative method to determine the pH at which maximum precipitation of casein is secured. In the beginning of this study, another problem was encountered in regard to turbidity of the filtrates.

To overcome this difficulty the following procedure was finally found to give satisfactory results: Have both milk and buffer below 15° C. when mixed. Shake thoroughly, then place in a water bath at about 60° C. for 15 minutes. Cool to 15° C. and filter at this temperature. It is important to have the filtrate at 15° C. when sampled for nitrogen determination. No. 589 S&S blue band filter paper was found most satisfactory. The slight turbidity sometimes noted was found to be due to milk fat.

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<sup>1</sup> *Mikrochemie*, 9, 269 (1931).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 80 (1936).

<sup>3</sup> *This Journal*, 18, 58, 407 (1935).

To study the *pH* at which maximum precipitation of casein occurred, a series of buffers was prepared from *N*/1 acetic acid and *N*/1 sodium hydroxide as follows:

|             | BUFFER  |         |         |         |         |         |
|-------------|---------|---------|---------|---------|---------|---------|
|             | 1       | 2       | 3       | 4       | 5       | 6       |
| Acetic acid | cc. 250 | cc. 250 | cc. 250 | cc. 250 | cc. 250 | cc. 250 |
| NaOH        | 50      | 75      | 100     | 110     | 125     | 150     |

The *pH* of the milk buffer mixture was determined by the quinhydrone electrode. The analytical procedure was carried out according to Method II, *Methods of Analysis*. A. O. A. C., 1930, 215.

The results are given in Tables 1 and 2, except for buffer No. 6, where the filtrates were too turbid to warrant analysis.

TABLE 1.—Casein nitrogen from various buffers

| MILK | CASEIN NITROGEN FROM EACH BUFFER |          |          |          |          |
|------|----------------------------------|----------|----------|----------|----------|
|      | 1                                | 2        | 3        | 4        | 5        |
| No.  | per cent                         | per cent | per cent | per cent | per cent |
| 1    | 0.371                            | 0.372    | 0.375    | 0.372    | 0.370    |
| 2    | 0.369                            | 0.370    | 0.370    | 0.367    | 0.367    |
| 3    | 0.369                            | 0.369    | 0.368    | 0.370    | 0.365    |
| 4    | 0.376                            | 0.376    | 0.377    | 0.374    | 0.374    |
| 5    | 0.370                            | 0.373    | 0.372    | 0.371    | 0.371    |
| 6    | 0.378                            | 0.381    | 0.383    | 0.383    | 0.379    |
| Av.  | 0.372                            | 0.374    | 0.374    | 0.373    | 0.371    |

TABLE 2.—*pH* values from various buffers

| Milk |       |       |       |       |       |
|------|-------|-------|-------|-------|-------|
| 1    | 4.173 | 4.343 | 4.454 | 4.454 | 4.688 |
| 2    | 4.223 | 4.336 | 4.505 | 4.605 | 4.717 |
| 3    | 4.203 | 4.355 | 4.516 | 4.569 | 4.700 |
| 4    | 4.123 | 4.320 | 4.431 | 4.544 | 4.598 |
| 5    | 4.108 | 4.254 | 4.483 | 4.580 | 4.668 |
| 6    | 4.214 | 4.316 | 4.512 | 4.604 | 4.707 |
| Av.  | 4.177 | 4.321 | 4.484 | 4.559 | 4.680 |

From the tables it will be seen that buffers 1 and 2, giving respective average *pH* values of 4.321 and 4.484, gave slightly higher casein values than did the other buffers. It was also noted that the filtrates from these were the clearest.

## ALBUMIN

A study of the pH for maximum yield of albumin has just been started. Indications are that both pH and dilution of the casein filtrate are important factors in determining the amounts of albumin.

## REPORT ON LACTOSE IN MILK

By E. R. GARRISON (University of Missouri,  
Columbia, Mo.), *Associate Referee*

As stated in the report for last year, a solution of mercuric iodide prepared and used according to the directions given in *Methods of Analysis*, A.O.A.C., 1930, 216, does not precipitate the proteins from milk sufficiently well to yield a clear filtrate. This formula, however, is in error since the original directions specified the use of 200 cc. of glacial acetic acid instead of 20 cc. as given in the present formula. A solution of mercuric iodide containing 33.2 grams of KI, 13.5 grams of  $\text{HgCl}_2$  in 200 cc. glacial acetic acid, and 640 cc. of  $\text{H}_2\text{O}$  was prepared and used for clarifying several samples of milk on which a lactose determination was later made. Different amounts of this solution (25, 30, 35, and 37.9 cc.) were used for clarifying the normal volume of milk (raw, herd milk) used. The filtrates obtained were clear in all cases, and the same lactose readings were secured with each amount of the precipitating agent used. However, when 25 cc. of the mercuric iodide solution was used, the milk coagulated slowly, yielding a very gelatinous precipitate that did not filter so fast as when larger amounts of the solution were used. For this reason it seems evident that 30 cc. of the mercuric iodide solution is necessary and also is an adequate amount to use for clarifying raw herd milk.

Lactose determinations were also made on milk somewhat abnormal in composition, *i.e.*, mastitis milk and milk obtained five or six days after freshening; the milk was clarified in duplicate determinations with 30 cc. of mercuric iodide solution and with 1 cc. of mercuric nitrate solution prepared as specified in *Methods of Analysis*. Both solutions yielded a clear filtrate at first with both types of milk, but the samples clarified with the mercuric nitrate solution became turbid upon standing. The lactose readings were essentially the same by both methods for the mastitis milk, but one sample of colostrum milk gave a lactose reading approximately 0.4 per cent lower by the mercuric nitrate method. This might indicate that the mercuric iodide solution may be preferable to the mercuric nitrate solution for use with abnormal milk, but an insufficient number of samples have been tested by both methods to warrant any general statement to this effect. Previous work demonstrated that



neither of these solutions yields satisfactory results with milk that is decidedly abnormal in composition.

It is therefore recommended<sup>1</sup> that the directions for the preparation of the mercuric iodide solution be changed to read "200 cc. of glacial acetic acid" instead of "20 cc. of glacial acetic acid" as is therein specified, and that the other directions be left unchanged.

It is also recommended that the directions for precipitation given in the second line of the next paragraph be changed to read as follows: "Add 1 cc. of the  $\text{Hg}(\text{NO}_3)_2$  solution or 30 cc. of the  $\text{HgI}_2$  solution (an excess of these reagents does no harm), fill to the mark, stopper, shake frequently for 15 minutes, filter through a dry filter, and polarize."

Protein removal is aided and a more flocculent precipitate is obtained by shaking the solution for 15 minutes. This prevents the lactose from being held in the hard, granular curd that forms when mercuric nitrate solution is used without sufficient shaking. This procedure tends to give a slightly higher lactose reading than is obtained when the present directions are followed.

It has also been found that the use of a larger volume of a more dilute solution of mercuric nitrate than is specified in *Methods of Analysis* gives more satisfactory results (higher lactose readings), but an insufficient amount of work has been done to warrant any definite recommendation for changes in the preparation of this solution at the present time.

## REPORT ON GELATIN

By G. A. RICHARDSON (Division of Dairy Industry, University of California University Farm, Davis, Calif.), *Associate Referee*

The Stokes method<sup>2</sup> for the qualitative detection of gelatin in milk, cream, and evaporated milk was accepted as official by this Association.<sup>3</sup> This method depends upon mercuric nitrate to cause precipitation in the dairy product so that picric acid when added to the filtrate therefrom fails to yield a precipitate except where gelatin was contained in the original product.

Richardson and Tarassuk<sup>4</sup> have recently pointed out that picric acid when added to the filtrate from the mercuric nitrate precipitation causes a flocculent precipitate to form in those cases where the milk proteins have undergone partial hydrolysis. This is especially true with sour milk, sour cream, evaporated milk, cultured buttermilk, cottage cheese, and those products to which rennet has been added during processing. In other words, the statement contained in the official test, "In the absence of

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 60, 92 (1936).

<sup>2</sup> *Analyst*, 22, 320 (1897).

<sup>3</sup> *Methods of Analysis*, A.O.A.C., 1930, 223.

<sup>4</sup> *This Journal*, 17, 314 (1934).

gelatin the filtrate will remain perfectly clear," is apparently true only in the case of raw sweet milk and raw sweet cream. The precipitate resulting from the action of the picric acid on the hydrolytic products of the proteins is flocculent and of non-adhering nature, and the serum is clear. In the case of gelatin the precipitate adheres tenaciously to the sides and bottom of the container and the serum is always turbid.

Richardson and Tarassuk<sup>1</sup> reported a modification of the Stokes method for the detection of gelatin in cultured buttermilk and cottage cheese. Use was made of the differentiation of the types of picric acid precipitate to predict the presence or absence of gelatin. In addition, trichloroacetic acid was employed to remove the derived proteins from the mercuric nitrate filtrate. In this manner perfectly clear solutions resulted from the addition of picric acid in the absence of gelatin and turbid solutions resulted in the presence of gelatin. Good agreement was found between the predicted and final conclusions, which in turn agreed with the known facts.

Additional experiments have been made to ascertain whether this modification could be applied to market creams and sterilized creams of known history, and to commercial sterilized creams the manufacturers of which gave their assurance that no gelatin had been added in the processing. The Stokes test was applied exactly as outlined in the official method. The modification consisted of the addition of one-half volume of cold 20 per cent trichloroacetic acid to an aliquot of cold filtrate from the mercuric nitrate precipitation. This mixture was shaken and allowed to stand at 8-10° C. for 12-16 hours with occasional shaking. At the end of this period the mixture was filtered cold, the filtrate was heated to 50° C., and an equal volume of hot (50° C.) saturated picric acid was added. This procedure is described in detail by Richardson and Tarassuk.<sup>1</sup> Tables 1 and 2 are replicas of the laboratory data sheets.

These results, obtained with the various types of cream, substantiate those already published<sup>1</sup> in connection with cottage cheese and cultured buttermilk. The Stokes method alone often leads to erroneous conclusions, but the introduction of trichloroacetic acid makes the method applicable to all the ordinary dairy products. In the experiments with cottage cheese and cultured buttermilk only one-half volumes of picric acid were used, but quite as satisfactory results were obtained with equal volumes.

Cottage cheese presents some slight difficulty, due largely to its high solids content, which necessitates the taking of special precautions in obtaining a satisfactory precipitation with mercuric nitrate.

It would seem necessary to amend the present method for the detection of gelatin in dairy products. An amended procedure that has been found to be applicable to all dairy products studied is recommended. It has been published.<sup>2</sup>

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<sup>1</sup> *Loc. cit.*

<sup>2</sup> *This Journal*, 19, 92 (1936).

TABLE 1.—*Observations recorded in testing commercial pasteurized creams for gelatin*

|  | 1   | 3  | 5  | 6   | 7   | 8  |
|--|---|--|--|---|---|--|
| Filtrate from mercuric nitrate (No. 1)   | Fairly clear  | Slightly turbid  | Fairly clear   | Slightly turbid   | Slightly turbid   | Same as 6  |
| Aliquot of filtrate No. 1 + equal vol. of saturated picric acid  | Fairly clear, very slight flocculent precipitate on standing. Serum clear | Colloidal turbidity. Sticky sediment on standing. Serum turbid | Slightly turbid flocculent precipitate on standing. Serum clear                | Colloidal turbidity. Precipitate on standing. Serum turbid  | Slightly turbid, flocculent precipitate on standing. Serum clear              |  |
| Prediction   | No gelatin  | Gelatin  | Rennet   | Gelatin   | Rennet  | Gelatin  |
| Aliquot of filtrate No. 1 chilled in ice water + $\frac{1}{2}$ vol. of cold 20% trichloroacetic acid. Allowed to stand 12-16 hours at 8-10° C. | Almost clear<br>Very slight precipitate                                   | Slightly turbid<br>Very slight precipitate                     | Slightly turbid<br>Slight precipitate  | Same as 5   | Same as 5   | Same as 5  |
| Conclusions  | No gelatin  | Gelatin  | No gelatin   | Gelatin   | No gelatin  | Gelatin  |
| Actual content   | Pasteurized table cream   | Pasteurized table cream + 0.2% gelatin                         | Pasteurized whipping cream 0.1 cc. 2% rennet/50 cc. cream, held cold overnight | Pasteurized whipping cream + 0.2% gelatin and 0.1 cc. 2% rennet/50 cc. cream, held cold overnight | Pasteurized table cream + 0.1 cc. 2% rennet/50 cc. cream, held cold overnight | Pasteurized table cream + 0.2% gelatin and 0.1 cc. 2% rennet/50 cc. cream, held cold overnight |

TABLE 2.—Observations recorded in testing commercial sterilized creams for gelatin

|   | 1   | 2                               | 3                                  | 4   | 5  |
|---|---|---------------------------------|------------------------------------|---|--|
| Filtrate from mercuric nitrate (No. 1)  | Fairly clear  | Same as 1                       | Same as 1                          | Fairly clear  | Fairly clear   |
| Aliquot of filtrate No. 1 + equal vol. of saturated picric acid   | Slightly cloudy<br>Slight flocculent precipitate settled out. Serum clear |                                 |                                    | Slight turbidity.<br>Sticky precipitate on standing. Serum turbid | Colloidal turbidity.<br>Sticky precipitate on standing. Serum turbid |
| Prediction  | No gelatin  | No gelatin                      | No gelatin                         | Gelatin   | Gelatin  |
| Aliquot of filtrate No. 1 chilled in ice water + $\frac{1}{2}$ vol. of cold 20% trichloracetic acid. Allowed to stand 12-16 hours with occasional shaking | Slightly cloudy.<br>Very slight precipitate                               | Same as 1                       | Same as 1                          | Same as 1   | Same as 1  |
| Filtrate from cold trichloracetic acid mixture heated to 50°C. + equal vol. hot (50°C.) saturated picric acid.  | Clear   | Clear                           | Clear                              | Slight colloidal turbidity  | Colloidal turbidity  |
| Conclusions   | No gelatin  | No gelatin                      | No gelatin                         | Gelatin   | Gelatin  |
| Actual content  | Sterilized light cream, 18% fat   | Sterilized table cream, 18% fat | Sterilized whipping cream, 36% fat | Sterilized whipping cream, 36% fat + 0.1% gelatin                 | Sterilized table cream 18% fat + 0.25% gelatin                       |

A paper, entitled "Comparison of Stokes Official Method with Certain More Recent Methods for Detecting Gelatin in Certain Dairy Products," by Carl S. Ferguson and Phileas A. Racicot, Boston, Mass., was presented (see p. 476).

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No report on sediment in cream and butter was given by the associate referee.

## REPORT ON NAVAL STORES

By F. P. VEITCH (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.), *Referee*

In connection with investigations of the Bureau of Chemistry and Soils and of the University of Florida on the influence of tree species, tree size, and season on the properties of rosin, the tentative methods of the Association for the analysis of rosin have been subjected to an extensive and critical study by A. P. Black and W. A. Dustin of the University of Florida, in which 173 samples of known origin and 42 samples of commercial rosin, including 13 commercial grades from X through B, were analysed. On 15 samples of these rosins determination of acid number and saponification number was also made by W. C. Smith of the referee's laboratory.

Since the work of Black and Dustin will be available shortly as a Department of Agriculture publication or in *This Journal*, it is not necessary to go into the details or submit actual analytical results. With the permission of these collaborators the referee will summarize the findings of this extensive and thoroughly done piece of work.

The report states that the tentative methods for the analysis of rosin, together with the suggested method for the determination of iodine number and the American Society for Testing Material method for softening point, are satisfactory with the exception of the method for determination of saponification number. The probable error in determination of acid number by a single analyst was found by Dustin to be not more than  $\pm .2$  of one per cent on duplicate samples. It is possible, however, that in the darker colored rosins the probable error of the determination will be greater. Smith's modification (use of the hand spectroscope in determining the end point) was found entirely adequate for determining the acid number of all grades. The method for determination of the petroleum ether-insoluble matter was also found satisfactory, the probable error in the hands of a single analyst being not more than  $\pm .07$  of one per cent. Dustin, however, recommends immediate filtration after solution of the rosin and finds a period for drying of 1.5 hours at 95–100° C. is sufficient when Gooch crucibles are used.

The method for ash was found to be satisfactory except that Dustin recommends that a larger sample than the 5 grams prescribed in the

method be used. He also recommends that a preliminary ashing be made over a Tirrell burner and that the ashing be completed in a muffle furnace for one hour at 900–1000° C.

The method for the determination of saponification number was found to be unsatisfactory, and while the referee is not prepared to recommend a final procedure it appears from results obtained in his laboratory and by Black and Dustin that one of the chief reasons for erratic results is the attack of the saponification flask by the alkali. There is a progressive attack apparently on most glassware. In this particular Pyrex seems to be very vulnerable and the error becomes greater with repeated use of the same flask. Dustin finds that this error can be largely overcome by first titrating the rosin soap solution to an end point, pouring out the soap solution, pouring 100 cc. of distilled water into the flask, and again titrating, adding the results to the original titration figures. When this procedure is followed, the results agree more closely. From his work Dustin concludes that the etching of flasks introduces a distinct error in the results and check analyses disagree. When used for saponification a definite number of times some flasks become etched more than others and the results obtained by using these mixed flasks are entirely unreliable. Over-titration with acid and immediate back-titration do not increase the accuracy of the results. The use of flasks that have been subjected to the same number of saponifications is quite necessary. Allowing the flasks to stand after initial titration and re-titrating increases accuracy and improves the checks obtained.

In the cooperative results on acid number of 15 samples of rosin, the maximum difference between the results of Smith and Dustin was 1.3. On saponification numbers the maximum difference was 3.4. The results on the acid number are considered satisfactory, but those on saponification number leave much to be desired and indicate that more study is required before concordant results may be expected in the hands of different analysts.

On account of the high cost to industrial laboratories of the 95 per cent ethyl alcohol specified for acid and saponification determinations the substitution of tax-free alcohols has been suggested. In an article in preparation for publication Smith shows that the results obtained on saponifying rosin with special denatured alcohol No. 30 as solvent are in close agreement with the results obtained with 95 per cent ethyl alcohol.

The referee is greatly indebted to Dr. Black and Mr. Dustin for permission to present to the Association in this brief way these results of their work.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That in the method for determining petroleum ether-insoluble matter the time of drying the crucible be reduced from 5 to 1.5 hours.

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<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 53 (1936).

(2) That for ash the quantity of sample used be 10 instead of 5 grams.

(3) That the study of the method for the determination of saponification number be continued.

(4) That the effect of substituting special denatured alcohol No. 30 for 95 per cent alcohol in the determination of acid and saponification number be studied.

No separate report on rosin was given by the associate referee. See report of Referee on Naval Stores.

No report on turpentine was given by the associate referee.

No report on paints was given by the referee. The recommendations he suggested were incorporated in the report of Subcommittee A, *This Journal*, 19, 51 (1936).

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No report on accelerated testing of paints was given by the associate referee.

## REPORT ON LEATHERS AND TANNING MATERIALS

By I. D. CLARKE (Industrial-Farm Products Division Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

The methods in the chapters on Leathers and Tanning Materials are essentially the same as the official and provisional methods of the American Leather Chemists' Association. These two chapters have been reviewed by R. W. Frey, F. P. Veitch, and the referee, with the object of making them conform more nearly to the methods of the A.L.C.A. A number of minor changes have been suggested, but they are of an editorial nature with one exception, that of the method, **XI, 25(a)**, for the extraction of raw or spent tanning materials. The proposed change specifies the use of a one liter instead of an 800 cc. boiling flask and refluxing for 14 hours with 500 cc. of water instead of for 5 and 9 hours with 250 cc. and 200 cc. of water, respectively. A slight change in the manipulation of transferring the charge to the extractor is also proposed.

## TUESDAY—AFTERNOON SESSION

### REPORT ON INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

By J. J. T. GRAHAM (Insecticide Division U. S. Food and  
Drug Administration, Washington, D. C.), *Referee*

No collaborative work was done this year on insecticides, fungicides, and caustic poisons. Last year a study was made of methods for the analysis of sodium hypochlorite solutions, calcium hypochlorite, bleaching powder, and chloramine-T. The results obtained were generally satisfactory, and some of the methods tested were adopted as official, first action. In view of the fact that the methods are well known it seemed unnecessary to subject them to further collaborative study.

It is recommended—

(1) That the methods for sodium hypochlorite, available chlorine, and chloride chlorine, sodium hydroxide and carbon dioxide, *This Journal*, **18**, 63–64 (1935), be adopted as official, final action.

(2) That the method for the determination of available chlorine in calcium hypochlorite and bleaching powder, *This Journal*, **18**, 64 (1935), be adopted as official, final action.

(3) That the methods for the determination of active chlorine, total chlorine, and sodium in chloramine-T (*This Journal*, **18**, 66 (1935), be adopted as official, final action.

Revision of Chapters VI and VII of *Methods of Analysis*.

At the request of the Chairman of the Board of Editors, *Methods of Analysis*, the referee reviewed the chapters on insecticides, fungicides and caustic poisons and makes the following recommendations:

(1) That the methods for the analysis of London purple, **32–41**, p. 42, be dropped. London purple has largely disappeared from the market, and there is very little need at present for these methods.

(2) That the volatilization method for the determination of fluorine in insecticides and fungicides, **146–149**, p. 59, be dropped. The method is long and tedious and does not give accurate results. The Willard-Winter method is much simpler in manipulation and is very accurate.

(3) That in the hydrogen peroxide method for the determination of formaldehyde, **130**, p. 56, the following statements be added at the end of the paragraph:

If the formaldehyde solution contains an appreciable quantity of free acid, titrate a separate portion and calculate the acidity as percentage of formic acid. Make correction for this acidity in calculating the percentage of formaldehyde.

Since derris and cubé and their derivatives are becoming more im-



portant as insecticides each year and large quantities are being used, it is recommended<sup>1</sup> that methods of analysis for these products be studied.

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No report on fluorine compounds was given by the associate referee.

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## REPORT ON SUGARS AND SUGAR PRODUCTS

By C. A. BROWNE (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.), *Referee*

Because of his long absence, due to attendance at the recent meeting of the International Sugar Cane Technologists at Brisbane, Australia, the Referee on Sugars and Sugar Products was unable during the past year to prepare and send out samples for a continuance of the collaborative work upon the clarification of pectin-containing food products for saccharimetric analysis. In the present report, therefore, he will limit himself briefly to certain observations upon the methods of saccharimetric analysis practised in the laboratories visited by him in the Hawaiian Islands, in the Fiji Islands, and in Australia.

A great deal of interest was manifested in the normal weight question. Usually the various sugar companies and chemists have followed their own preferences in the selection of polarizing apparatus, instruments of German, Austrian, Czechoslovakian, French, English, and American manufacture being used with the usual variation in saccharimeter scales and normal weights. This latitude of usage had also been observed by the referee in his visits to the sugar-producing countries of the West Indies, of Europe, and of the Near East during previous years. In the numerous sugar factories and refineries of the Colonial Sugar Refining Company, in the Fiji Islands, and in Australia, the bidecimal normal weight of 20.00 grams (the so-called International Scale of Sidersky and Pellet) is generally employed. This normal weight is also used in the island of Mauritius, in the sugar factories of Egypt, and in various individual laboratories of different countries where chemists have recognized the advantages of a decimal normal weight. These advantages have been indicated by the referee and other writers upon the subject and need not be mentioned again at this time.

In the sugar factories of the Hawaiian Islands and in many of the privately owned and cooperative sugar factories of Australia the old Ventzke saccharimeter with a rotation value for its 100° point of 34.657 angular degrees for sodium light is very largely employed, although there are also in use a few of the newer instruments, equipped with the Bureau

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 46 (1936).

of Standards scale, having a rotation value for its 100° point of 34.62 angular degrees for sodium light.

In France and the French Colonies, in several Latin countries, and in numerous individual laboratories throughout the world the French saccharimeter scale, with a rotation value for its 100° point of 21.666 angular degrees of sodium light, is generally used. The normal weight for this scale is usually given as 16.29 grams, but a little uncertainty still prevails as to its exact value so that a redetermination of this constant is felt to be necessary by many chemists.

These observations and the recent published comments of Parr, Sallard, and others upon the normal weight question indicate the impossibility of obtaining general international agreement among the chemists of the world upon the particular type of polarization apparatus which shall be employed in the analysis of sugar products. This Association has wisely refrained in its methods of analysis from prescribing any particular type of saccharimetric, refractometric, or densimetric apparatus. The only requirement is that accuracy shall be obtained under the conditions pertaining to each individual type of instrument. Normal weights and quartz plates for scale verification must, therefore, correspond to the particular type of saccharimeter which the analyst employs.

The referee observed also during the recent tour a considerable degree of latitude in the preferences of chemists for particular methods of sugar analysis and here again as in the choice of saccharimeter scales there can be no immediate hope of securing absolute international uniformity. All that can be expected is a general agreement upon fundamentals without requiring a rigid adherence to details that do not affect the accuracy of the final results.

Attempts were made some years ago to establish an international bureau in a certain European capital for prescribing the methods which shall be used in determining the sugars, fats, proteins, acids and other organic and mineral constituents of the foods and agricultural products which are handled in the trade transactions of different countries. The establishment, however, of such a bureau in one particular national center aroused such suspicions of self-interest among chemists of other countries that the attempt was doomed to failure. It is not to be expected, or even to be desired, that the methods of this Organization shall be accepted as official in other countries. In the same way the methods recommended in other countries or by international organizations should not be accepted as official by this Association until they have been tested and approved by its referees.

It is recommended<sup>1</sup> for the second and final reading that the table of conversion factors for different saccharimeter scales, recommended last year, be published in the next edition of *Methods of Analysis*. These

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936).

factors, like the values for atomic weights, are of course subject to such revision as future redeterminations of these constants may dictate.

It is also recommended that the study of the effect of clarifying agents upon the polarization of food products be extended to the examination of jellies and other pectin-containing materials.

No report on honey was given by the associate referee.

The paper, "Observations on Adulteration of Honey with Commercial Invert Sugar," by R. E. Lothrop, was published in *This Journal*, 19, 338 (1936).

## REPORT ON MAPLE PRODUCTS

By J. F. SNELL (Macdonald College, Province of Quebec, Canada), *Associate Referee*

Attention was given to the two recommendations (4 and 5) approved by the Association at the 1934 meeting<sup>1</sup> and to a device suggested by C. H. Jones of the Vermont Agricultural Experiment Station for amelioration of the washing of the Canadian lead precipitate.

## EXAMINATION FOR PLANT TISSUES

Commercial sugars derived from sugar cane or sugar beet sometimes contain plant particles. Such particles may be collected for microscopic examination by treating the sugars with water and centrifuging the solutions so obtained. Lancaster<sup>2</sup> suggested that by means of such an examination it might be possible to detect the adulteration of maple sirup and sugar with cane and beet products. Ernst Artschwager of the Bureau of Plant Industry, U. S. Department of Agriculture, an eminent authority on the anatomy of the sugar cane and the sugar beet, kindly examined the centrifuged residue of two sugar cane solutions and found no plant cells or tissues of any sort. He also examined in a similar manner ten of the pure maple sirups collected for the collaborative work of 1934, some of Canadian and some of United States origin. In these he found crystals of various types and sizes, tissues of maple origin, fungous spores and pollen grains, but, naturally, no trace of corn or cane tissue. F. S. Thatcher of the Macdonald College Department of Plant Pathology also centrifuged solutions of commercial cane sugar, white and brown, and of maple sirups, and found in both yeast cells, bacteria, certain fungous spores, and fragments of fungus mycelium. In addition he found fragments of plant tissue, larger and more abundant in the maple sirup than in the

<sup>1</sup> *This Journal*, 18, 46 (1935).

<sup>2</sup> *Ibid.*, 9, 155-6 (1926).

cane sugar. These included epidermal cells; parenchyma cells; and pitted, spiral, and thickened xylem cells or particles of these. In the cane sugar the xylem cells were in greater relative proportion than in the maple sirup, but comparison of these elements with fragments of maple leaves pulverized in a mortar failed to reveal any decisive difference. The results on the cane sugar, though confirmatory of those found in Lancaster's laboratory, were at variance with those reported by Artschwager and also with the general belief of sugar refiners that no plant particles of more than colloidal dimensions could possibly pass through the numerous filterings of the refining process. It appeared possible that the plant fragments obtained from commercial samples of sugar might be of fortuitous origin. Through the courtesy of the Canada and Dominion Sugar Company, samples of yellow and of white cane sugar, obtained directly from the refinery, were subjected to the same examination. Even in these, minute fragments of plant material were detected in very small quantity by Thatcher, but they were too small to suggest a definite plant source.

#### EXAMINATION OF MAPLE FLAVOR CONCENTRATES AND IMITATIONS

With the collaboration of the Chemistry Division of the National Research Council of Canada, a collection of materials offered for sale as true or as imitation maple flavors was attempted. Up to the present there have been obtained five preparations from genuine maple sirup, three others labelled as true maple flavor, and nine admitted imitations. The preparations from genuine maple sirup are two prepared by Les Producteurs de Sucre d'Erable de Québec, Levis, Québec, according to Lund and Anthony's U. S. Patent 1,957,465, May 8, 1934, namely by centrifuging the molasses from crystals slowly formed in a sirup evaporated to 80° Brix; two prepared by Leo Skazin in the National Research Council Laboratories at Ottawa from a similarly concentrated sirup to which alcohol had been added (U. S. Patent 1,961,714, June 5, and Canada Pat. 342,570, June 26, 1934); and one prepared by Fritzsch Bros., New York, according to Sale and Wilson's U. S. Patent 1,642,709, Sept. 20, 1927, by a process in which the sugar is precipitated from a sirup of 28° Brix by addition of a solution of barium hydroxide and removal of the excess of barium by treatment with sulfuric acid. It is claimed that the products of Les Producteurs de Sucre d'Erable de Québec, designated Canada Strong and Canada Strong XXX, have, respectively,  $2\frac{1}{2}$  and 4 times, and those of the National Research Council,  $2\frac{1}{2}$  and 8 times, the concentration of flavor contained in ordinary maple sirup. The makers of Fritzbro True Maple Concentrate claim a much greater concentration, such that  $1\frac{3}{4}$  oz. will flavor 1 gallon of sugar sirup, which would mean that the preparation contains 73 times more flavor in a given volume than does maple sirup.

The associate referee's examination of these samples is designed to in-

clude: (1) application of the usual maple sirup analytical methods to sirups prepared from the flavor concentrates, or to the concentrates direct; (2) tests for, or determinations of, constituents likely to be present in the imitations, among which may be mentioned vanillin, coumarin, caffein, oil of lovage, fenugreek, glycerol, and alcohol; (3) comparison of color tests obtainable with the pure and imitation flavors; and (4) comparison of the intensity and quality of flavor of sirups prepared from the various concentrates. Results cannot be reported at present, as the work is far from complete.

#### DEVICE TO AMELIORATE WASHING OF THE CANADIAN LEAD PRECIPITATE

In a letter dated April 5, C. H. Jones of the Vermont Agricultural Experiment Station, one of the pioneers in the chemical study of maple products, reported a procedure often used in his laboratory to prevent the formation of fissures in the Canadian lead precipitate which, serving as open channels, interfere with the thorough washing of the precipitate in the crucible.<sup>1</sup>

When the precipitate has been allowed to settle the prescribed two hours,<sup>2</sup> a weighed quantity of dry asbestos, appropriate to the amount

TABLE 1.—*Canadian lead values as determined without and with the asbestos filter aid*

| SAMPLE<br>NO. | BY I. PUDDINGTON   |         |                 |         | BY 1934 COLLABORATORS WITHOUT<br>FILTER AID |      |      |
|---------------|--------------------|---------|-----------------|---------|---|------|------|
|               | WITHOUT FILTER AID |         | WITH FILTER AID |         | J   | B    | F    |
|               | DUPLICATES         | AVERAGE | DUPLICATES      | AVERAGE |   |      |      |
| 17            | 2.480              |         | 2.494           |         |   |      |      |
|               | 2.460              | 2.470   | 2.490           | 2.492   | 2.54  | 2.27 | 2.73 |
| 5             | 3.512              |         | 3.538           |         |   |      |      |
|               | 3.560              | 3.536   | 3.524           | 3.531   | 3.92  | 3.48 | 4.08 |
| 15            | 6.152              |         | 6.228           |         |   |      |      |
|               | 6.130              | 6.141   | 6.142           | 6.175   | 7.76  | 6.30 | 7.92 |
| By A. Conlin  |                    |         |                 |         |   |      |      |
| 1             | None               | 3.06    | None            | 3.07    | Vermont No. 1 sirup                         |      |      |
| 2             | None               | 3.32    | None            | 3.27    | Vermont No. 1 sirup                         |      |      |
| 3             | None               | 3.45    | None            | 3.50    | Vermont No. 1 sirup                         |      |      |
| 4             | None               | 4.27    | None            | 4.18    | Vermont No. 3 & 2 sirup                     |      |      |

of precipitate (0.5 gram is usually sufficient), is stirred in with a rod, and the mixed precipitate and asbestos are transferred to the prepared Gooch. This procedure, Jones also finds, accelerates the filtration, though the

<sup>1</sup> Fowler and Snell, *Ind. Eng. Chem. Anal. Ed.*, 1, 8-12 (1929).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1930, 393, 119.

time required for oven-drying may be longer than with the usual procedure. Augustus Conlin, Chemist of the Cary Maple Sugar Company, St. Johnsbury, Vermont, has also found this procedure to be advantageous.

Table 1 gives results of comparative experiments made by Ira Puddington, Macdonald College, upon three of the 1934 A.O.A.C. samples. The quantity of dried asbestos used was always about 0.5 gram. To obviate making an additional weighing the dried asbestos was weighed in the dry, prepared Gooch. It was then transferred to the 50 cc. beaker used in the precipitation, stirred in, and allowed to settle for 5 minutes before filtration. In the same table are shown results obtained by Conlin on four Vermont sirups. The results are satisfactory, and the procedure is recommended to other analysts for trial.

#### SUMMARY

1. Examination of concentrates and imitations of maple flavor is in progress.

2. In the detection of adulteration of maple products with refinery products from sugar cane, microscopic examination of centrifugal sediments of solutions has not proved useful.

3. Jones' device of adding asbestos to the Canadian lead precipitate before filtration is useful as an aid to filtration and effective washing.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That work on maple flavor concentrates and imitations be continued.

(2) That the modification of the official procedure for the determination of Canadian lead value suggested by C. H. Jones, viz., the addition of asbestos to the precipitate before filtration, be subjected to collaborative study.

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### REPORT ON DRYING, DENSIMETRIC AND REFRACTOMETRIC METHODS

By C. F. SNYDER (National Bureau of Standards,  
Washington, D.C.), *Associate Referee*

It is suggested that the following editorial change be made in the refractometric method, *Methods of Analysis, A.O.A.C.*, 1930, p. 365, which will incorporate the two additions to the method that have been adopted by the Association, *This Journal*, 15, 79 (1932); 17, 46 (1934).

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936)

## REFRACTOMETRIC METHOD—OFFICIAL

(Applicable only to liquid samples containing no undissolved solids)

Determine the refractometer reading of the solution at 20°C. and obtain the corresponding percentage of dry substance from either the direct reading, if a sugar refractometer is used, or from Table 6, XLII, if the instrument is calibrated in terms of refractive index. Circulate water at a constant temperature, preferably 20°C., through the jackets of the Abbé refractometer or through the trough of the immersion instrument for a sufficient time to allow the temperature of the prisms and of the sample to reach an equilibrium. Continue circulation during observations, taking care that the temperature is kept constant. If the room temperature is appreciably higher than 20°C. or if the humidity causes condensation of moisture on the exposed faces of the prisms, maintain the circulating water at room temperature. If the refractometer reading is obtained at a temperature other than 20°C., correct the results according to Table 7, XLII. If the solution is too dark . . .

The work of the past year was on the determination of the refractive indices of dextrose solutions. Solutions of chemically pure dextrose of various concentrations up to 85 per cent were prepared, and the refractive indices were measured on a carefully calibrated Abbé type refractometer. The usual precautions were taken to insure accurate temperature control at the standard temperature of 20°C. The mean values at each concentration were plotted on a large-scale graph and a smooth curve drawn. By means of a graphic interpolation a table of indices for even per cent concentrations was constructed.

H. C. S. de Whalley, in the *International Sugar Journal*, September 1935, reports the results of his investigation of the refractometric estimation of dissolved solids in sugar sirups containing invert sugar. He points out the invert sugar solutions prepared by dissolving equal parts of dextrose and levulose give low results when read on the refractometer, and converted by means of the sucrose tables. He therefore recommends that a correction be applied to the refractometer solids of 0.022 for each 1 per cent invert in order to give true solids.

It may be pointed out that the de Whalley values for invert sugar are in satisfactory agreement with the mean of the above-mentioned dextrose values and the levulose values of Jackson and Mathews.

Another interesting observation of de Whalley was that the values obtained on invert sugar solutions prepared by dissolving dextrose and levulose differed from values obtained on solutions prepared by inverting sucrose.

RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That the refractive indices of invert sugar solutions be determined.
- (2) That the change in refractive indices with change of temperature be determined for such products as invert sugar solutions, table sirups, etc.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936).

A paper, entitled "Constant Temperature Apparatus for Refractometers," was presented by P. T. Kirwan of Baltimore, Md.

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No report on polariscopic methods was given by the associate referee.

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No report on chemical methods for reducing sugars was given by the associate referee.

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## REPORT ON LEAD PRECIPITATE

By F. W. ZERBAN (New York Sugar Trade Laboratory,  
New York, N. Y.), *Associate Referee*

In the report presented before this Association last year<sup>1</sup> it was shown that clarification with lead subacetate solution increases the true direct polarization of raw cane sugars, that the use of Horne's dry lead method reduces the plus error to a large extent, and that dry mercuric acetate slightly decreases the true polarization. In accordance with the recommendation made at that time the effect of mercuric acetate on the rotation of sucrose, dextrose, and levulose, and of mixtures of these sugars alone or after the addition of non-sugars, was studied further.

Neuberg and Ishida<sup>2</sup> investigated the influence of mercuric acetate on the polarization of the three sugars named above, but at concentrations of only 2.5 to 3.5 per cent. They found that under these conditions the rotation of the sugars is not measurably affected, and they pointed out that this offers a great advantage over lead subacetate, because this reagent materially reduces the levorotation of levulose and converts the slight levorotation of asparagine or aspartic acid into strong dextrorotation, whereas mercuric acetate precipitates these amino compounds.

The experiments of Neuberg and Ishida were repeated in this Laboratory, by C. A. Gamble and J. E. Mull, with normal weight solutions of the three sugars, that is 26.000 grams of sucrose, 32.231 grams of dextrose, and 18.407 grams of levulose in 100 ml. solution. The dextrose was kindly furnished by H. Berlin, of the Corn Products Refining Co.; the levulose was a commercial preparation of high purity. Mercuric acetate, at the rate of 0.0, 0.5, 1.0, 1.5, and 2.0 grams, was dissolved in each solution before the volume was completed, and in another series after the sugar solution had been diluted to the mark, at 20° C. It was found, however, that not more than 0.5 gram of mercuric acetate could be dissolved in the normal dextrose solution. The larger amounts dissolved upon warming, but the excess salt crystallized out upon cooling to 20° C. For this reason another series of experiments was made with half-normal weight solutions of

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<sup>1</sup> *This Journal*, 18, 178 (1935).

<sup>2</sup> *Z. Ver. deut. Zucker-Ind.*, 61, 1113 (1911).



dextrose, and only half the quantities of mercuric acetate; the observed rotations were doubled. While these results are not directly comparable with the others, they nevertheless clearly show the tendency in the effect of the clarifying agent. The solutions were read in a saccharimeter at 20° C. Owing to differences in rotation dispersion the dextrose and levulose solutions were very difficult to read, and it was necessary to double the concentration of the bichromate solution used as a light filter. The results are shown in Table 1.

TABLE 1.—*Effect of mercuric acetate on polarization of sugars*

|                             | QUANTITY OF MERCURIC ACETATE ADDED (GRAMS) |        |        |        |        |
|-----------------------------|--|--------|--------|--------|--------|
|                             | 0 00                                       | 0.5    | 1.0    | 1.5    | 2.0    |
| A. Before making up to mark |  |        |        |        |        |
| Sucrose                     | 99.90                                      | 99.88  | 99.88  | 99.88  | 99.88  |
| Dextrose, normal wt.        | 99.75                                      | 99.72  | —      | —      | —      |
| Dextrose, half-normal wt.   | 98.90                                      | 98.75  | 98.65  | 98.55  | 98.45  |
| Levulose                    | -98.95                                     | -99.03 | -99.10 | -99.15 | -99.30 |
| B. After making up to mark  |  |        |        |        |        |
| Sucrose                     | 99.90                                      | 99.73  | 99.60  | 99.48  | 99.35  |
| Dextrose, normal wt.        | 99.75                                      | 99.63  | —      | —      | —      |
| Dextrose, half-normal wt.   | 98.90                                      | 98.75  | 98.60  | 98.50  | 98.30  |
| Levulose                    | -98.95                                     | -99.03 | -98.78 | -98.70 | -98.60 |

The mercuric acetate, added before the volume is completed, has practically no effect on the rotation of sucrose; the polarization is the same, whether 0.5 or 2 grams are used. But the mercuric acetate materially decreases the dextrorotation of the dextrose, and increases the levorotation of levulose, which is equivalent to a decrease in dextrorotation. The combined effect on dextrose and levulose is thus additive, and opposite to that produced by lead subacetate.

If the mercuric acetate is added after the solution has been made up to the mark, the increase in the volume of the solution, when no precipitate is produced, is evidenced by a decrease in the dextrorotation of sucrose or dextrose, and in the levorotation of levulose. Only in the case of levulose with 0.5 gram mercuric acetate was the increase in volume apparently not sufficient to overcome the increase in levorotation produced by the salt.

When mercuric acetate is added to the solution of a raw sugar before the volume is completed, a precipitate is produced, and this raises the resulting polarization, just as lead subacetate does. The volume error may be corrected by first completing the volume and then adding a quantity of mercuric acetate just sufficient to leave no excess of mercury in the solution. Any excess added, either before or after completing the volume, tends to decrease the dextrorotation of the sugar mixture. This

explains, in a general way, the results obtained with mercury clarification last year, when 0.4 gram of the dry acetate was added after making up to the mark. In the case of the Cuban sugars, this quantity was evidently just sufficient for the mercury to be completely precipitated, and the average error was only  $-0.002$ . With the Hawaiian sugars, of much higher purity than the Cuban, a part of the mercury salt remained in solution, and depressed the average true polarization by  $0.059$  degree. For the sugars from other sources the minus error was somewhere between these two extremes, and averaged  $-0.029$ , compared to a plus error of  $0.054$  with dry lead subacetate clarification.

In order to obtain a still clearer picture of the effect of clarifying raw sugar solutions with mercuric acetate, a further study was made with mixtures of the three sugars and of non-sugars occurring in raw cane sugars. The plan was similar to that used by Zerban and Gamble<sup>1</sup> in their study on the effect of lead subacetate.

I. The following solutions were prepared:

|     | <i>Sucrose</i> | <i>Parts of Dextrose</i> | <i>Levulose</i> |
|-----|----------------|--------------------------|-----------------|
| (a) | 96             | 0.5                      | 0.5             |
| (b) | 96             | 0.25                     | 0.75            |
| (c) | 96             | 0.75                     | 0.25            |
| (d) | 96             | 1                        | 1               |
| (e) | 96             | 0.5                      | 1.5             |
| (f) | 96             | 1.5                      | 0.5             |

II. Same as I, (a) to (f), plus 0.25 part each of potassium sulfate and sodium aconitate.

III. Same as I, (a) to (f), plus 0.5 part each of potassium sulfate and sodium aconitate.

IV. Same as I, (a) to (f), plus 0.25 part each of potassium sulfate and sodium aspartate.

Solutions containing the normal weights of the sugar mixtures, plus the non-sugars added in II to IV, were prepared in 100 ml. flasks. In one series, 0.3 and 0.6 gram, respectively, of mercuric acetate was added in the form of a solution before the volume was completed. In the other series, the same quantities of mercuric acetate were added in solid form, after the solution had been made up to the mark. After thorough shaking, the mixtures were filtered, and 25 ml. of filtrate was discarded. The remainder of the filtrate was polarized at  $20^{\circ}$  C. in a 200 ml. tube. Unclarified solutions were polarized in each case, as a check, and the average differences between the unclarified and the clarified solutions, found by C. A. Gamble and J. E. Mull, are shown in Table 2.

<sup>1</sup> *Facts About Sugar*, 28, 180 (1933).

TABLE 2.—Effect of mercuric acetate on polarization of artificial raw sugars

| Sugar Mixture | A. Before making up to mark |        |   |        |  |        |
|---------------|-----------------------------|--------|---|--------|--|--------|
|               | I. Sugars only              |        | II. Sugars plus 0.25% K <sub>2</sub> SO <sub>4</sub> , 0.25% Na acon. |        | III. Sugars plus 0.5% K <sub>2</sub> SO <sub>4</sub> , 0.5% Na acon. |        |
|               | 0.3                         | 0.6    | 0.3   | 0.6    | 0.3  | 0.6    |
|               | gram                        |        | gram  |        | gram   |        |
|               | Hg (AcO) <sub>2</sub>       |        | Hg (AcO) <sub>2</sub>   |        | Hg (AcO) <sub>2</sub>  |        |
| (a)           | +0.05                       | +0.05  | +0.05   | -0.02  | -0.02  | +0.13  |
| (b)           | -0.03                       | -0.05  | +0.05   | +0.05  | +0.03  | +0.15  |
| (c)           | -0.03                       | 0.00   | 0.00  | +0.03  | 0.00   | +0.15  |
| (d)           | -0.05                       | -0.05  | +0.02   | 0.00   | +0.03  | +0.17  |
| (e)           | 0.00                        | -0.03  | +0.02   | +0.05  | 0.00   | +0.15  |
| (f)           | -0.05                       | -0.03  | -0.05   | -0.02  | -0.03  | +0.15  |
| Averages      | -0.018                      | -0.018 | +0.015  | +0.015 | +0.002   | +0.142 |
|               |                             |        |   |        |  | +0.162 |
|               | B. After making up to mark  |        |   |        |  |        |
|               |                             |        |   |        |  |        |
|               | 0.3                         | 0.6    | 0.3   | 0.6    | 0.3  | 0.6    |
|               | gram                        |        | gram  |        | gram   |        |
|               | Hg (AcO) <sub>2</sub>       |        | Hg (AcO) <sub>2</sub>   |        | Hg (AcO) <sub>2</sub>  |        |
| (a)           | -0.13                       | -0.20  | -0.07   | -0.10  | -0.12  | +0.08  |
| (b)           | -0.06                       | -0.14  | +0.03   | 0.00   | -0.02  | +0.10  |
| (c)           | -0.15                       | -0.23  | -0.05   | -0.05  | -0.10  | +0.10  |
| (d)           | -0.15                       | -0.25  | -0.10   | -0.13  | -0.17  | +0.12  |
| (e)           | -0.13                       | -0.23  | -0.03   | -0.10  | -0.07  | +0.10  |
| (f)           | -0.15                       | -0.25  | -0.12   | -0.15  | -0.15  | +0.15  |
| Averages      | -0.128                      | -0.217 | -0.057  | -0.090 | -0.090   | +0.108 |
|               |                             |        |   |        |  | +0.065 |

In series A, where the clarifying agent was added before completion of the volume, the differences between the clarified and unclarified solutions, in the absence of amino compounds (Mixtures I to III), were generally very slight, and averaged  $-0.008$ ; with 0.3 gram of mercuric acetate they averaged 0.000, with 0.6 gram  $-0.014$ . These averages are much smaller than, and opposite in direction to, those found for clarification with corresponding quantities of lead subacetate solution, viz.  $+0.050$ ,  $+0.035$ , and  $+0.066$ , respectively.

In the absence of ash, there is a small average decrease,  $-0.018$ , of the same order of magnitude as would be expected from the results shown in Table 1. The addition of 0.5 per cent of ash converts this decrease into a slight average increase,  $+0.015$ , showing the effect of the volume of the precipitate. When 1 per cent of ash is added the volume error is overshadowed by another effect: with both 0.3 and 0.6 gram of mercuric acetate, there is no further increase, but actually a decrease in the rotation compared to that when only 0.5 per cent of ash is present. This is probably due to the fact that the larger quantity of potassium and sodium acetate formed from the larger quantity of ash has a greater depressing effect on the polarization than the mercuric acetate, and that this effect more than compensates for the volume of the precipitate.

In those experiments where aspartate was substituted for the aconitate, there is a decided increase in polarization due to clarification, because the aspartic acid is precipitated as the mercury salt, and besides, levorotation of the sodium aspartate is eliminated thereby. But the increase in dextrorotation is still much smaller than when lead subacetate is used for clarification. Although in the latter case the aspartic acid is not precipitated, the levorotation of the sodium aspartate is converted into the strong dextrorotation of the soluble lead salt. In the mixtures listed under I to III the influence of variations in the quantities of dextrose and levulose is too small to be detected, but in the mixtures containing aspartate (IV), there are larger differences between the clarified and unclarified solutions in the presence of 2 per cent than in that of 1 per cent total reducing sugars.

In Series B, with dry clarification after completion of the volume, the effect of the increase in the volume of the sugar solution by the addition of the mercury salt is clearly apparent in the mixtures containing only sugars (I). The depressing effect on the dextrorotation is less when the levulose is high (b and e) than when it is low (c and f). This was to be expected from the results shown in Table 1.

In the mixtures containing ash (II to IV), the dry clarification also produced generally lower polarizations than did wet clarification, owing partly to correction for the volume of the precipitate produced, and partly to dilution from the excess of clarifying agent used.

In the presence of 0.5 per cent of ash (II) the influence of the volume

of precipitate is well marked, the average depression in the dextrorotation being 0.071 and 0.127, respectively, smaller than in the absence of ash. In the experiments with 1 per cent ash (III), the same phenomenon is noted as in Series A. Instead of a greater increase in the dextrorotation, as would be expected from a larger volume of precipitate, there is a smaller one than with 0.5 per cent of ash, and this must be explained in the same way as shown in the discussion of the results of Series A.

When aspartate is substituted for the aconitate (IV), the reduction in the plus error by dry instead of wet clarification is also clearly indicated. But a plus error still remains, owing to the elimination of the levorotatory sodium aspartate. The average increase in the rotation over the unclarified solution is again larger when 2 per cent of reducing sugars are present than when only 1 per cent has been added.

The quantity of aspartate used in these experiments was larger than is usually found in raw sugars. With smaller amounts the increase in dextrorotation with wet clarification would have been less, and the error obtained by dry clarification would have been nearer zero.

The results of this investigation clearly show that, like in the case of lead subacetate, there are many factors involved in clarification with mercuric acetate: the quantity used; whether it is added before making up to the mark, or in dry form after completion of the volume; the volume of the precipitate produced; the precipitation and change in rotation of amino compounds; the effect on the rotation by salts which are formed by the interaction between the mercuric acetate and the salts naturally present in the sugar. From the practical standpoint, it must also be considered that the precipitate produced by mercuric acetate is much smaller than that obtained with lead subacetate, and that the solutions do not filter so well.

It may be concluded that neither dry lead subacetate nor mercuric acetate, used alone, is completely satisfactory for clarification of raw sugars. But since dry lead subacetate tends to produce a plus error, and mercuric acetate a minus error, it should be possible, as stated in the previous report, to find a combination of the two reagents in such proportions that the average polarization of raw sugars from various sources would coincide very closely with the average true polarization. It is recommended<sup>1</sup> that this question be further investigated with actual raw sugars.

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No report on vinegars was given by the referee.

No report on ash in vinegar was given by the associate referee.

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936).

## REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The two chapters in *Methods of Analysis* which come under this refereeship, namely Flavoring Extracts and Beverages (non-alcoholic) and Concentrates, have been carefully reviewed, and a number of editorial changes, which do not affect the underlying principles of the methods, are being made.

## FLAVORING EXTRACTS

The referee has found no method for the determination of total aldehydes which gives promise of improvement over the present official methods.

At the request of the Alcohol Tax Unit, additional collaborative work was done on the determination of oil in flavoring extracts by the method adopted as tentative in 1933. The purpose of this work was to extend the method to other extracts. The samples this year were made from fresh oils purchased from reputable manufacturers, and the results obtained indicate that the previous work with peppermint extracts was at fault because of the quality of oil used in their preparation.

An extract was prepared this year from an oil that was used in 1933. As the results obtained on the two extracts were quite different, the referee gave up further determinations requiring saturated calcium chloride solution and returned to the original procedure. Twelve extracts were prepared for collaborative work. Allspice, caraway, and peppermint oils were obtained from two manufacturers, and two strengths of extract were prepared by the referee from each oil and submitted to the Alcohol Tax Unit. The results obtained are reported in Table 1. However, it was

TABLE 1.—*Oil in flavoring extracts*

| EXTRACT    | OIL |   | PRESENT         | BURRITT         | OIL FOUND       |                 |
|------------|-----|---|-----------------|-----------------|-----------------|-----------------|
|            |     |   |                 |                 | VALAER          | MALLORY         |
|            |     |   | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> | <i>per cent</i> |
| Caraway    | A   | 1 | 3.00            | 3.2             | 3.0             | 3.0             |
|            | B   | 1 | 2.60            | 2.6             | 2.4             | 2.6             |
|            | C   | 2 | 3.30            | 3.4             | 3.4             | 3.4             |
|            | D   | 2 | 3.05            | 3.0             | 3.0             | 3.0             |
| Allspice   | E   | 1 | 2.50            | 2.4             | 2.2             | 2.2             |
|            | F   | 1 | 3.40            | 3.6             | 3.6             | 3.4             |
|            | G   | 2 | 2.80            | 2.7             | 2.6             | 2.5             |
|            | H   | 2 | 3.20            | 3.2             | 3.2             | 2.8             |
| Peppermint | I   | 1 | 2.40            | 2.2             | 2.2             |                 |
|            | J   | 1 | 3.60            | 3.4             | 3.4             |                 |
|            | K   | 2 | 2.80            | 3.0             | 2.8             |                 |
|            | L   | 2 | 3.20            | 3.4             | 3.1             |                 |

found that in determining peppermint oil and allspice (pimiento) oil, the column of oil should be read from the bottom of the meniscus at its top rather than at the top of this meniscus, as is the case with all other extracts.

The referee considers that these results are sufficiently accurate for a rapid method and that they warrant the inclusion of these three extracts in the list to which this method may be applied.

#### BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

The referee has felt for some time that the organoleptic examination of these products has not been sufficiently stressed by analysts. Frequently, such examination discloses important facts regarding their composition and forms a guide to the analyst in his work. He should, therefore, be directed to observe the appearance, color, odor, and flavor of these products.

A uniform procedure is being recommended for the preparation of sample for the determination of the fruit acids. It will appreciably expedite the work when the several fruit acids are to be determined upon the same sample.

A procedure for the determination of benzaldehyde in non-alcoholic beverages and concentrates,<sup>1</sup> similar to that for cordials and liqueurs, was submitted to collaborative work on a flavor, two sirups, and two beverages, made up for this purpose. The results, which are considered good, are given in Table 2.

TABLE 2.—*Benzaldehyde*

| SAMPLE     | PRESENT     | FOUND                | COLLABORATOR                |
|------------|-------------|----------------------|-----------------------------|
|            | mg./100 cc. | mg./100 cc.          |                             |
| Flavor B   | 196         | 196<br>194           | Wilson                      |
| Sirup E    | 9.8         | 9.6<br>9.8<br>9.5    | Winkler<br>Wilson<br>Wilson |
| Sirup F    | 39.1        | 36.9<br>39.0<br>40.4 | Winkler<br>Wilson<br>Wilson |
| Beverage G | 1.4         | 1.1<br>1.3<br>1.4    | Winkler<br>Wilson<br>Wilson |
| Beverage H | 5.6         | 5.4<br>5.6<br>5.6    | Winkler<br>Wilson<br>Wilson |

<sup>1</sup> *This Journal*, 19, 75 (1936).

The procedure for the detection of gamma-undecalactone in cordials and liqueurs is equally applicable to non-alcoholic beverages and concentrates.

The following methods are therefore recommended<sup>1</sup> for adoption as tentative: Physical Examination, Characteristic Acids, Tartaric Acid, Citric Acid, Malic Acid, Benzaldehyde and Gamma-Undecalactone. These methods were published in *This Journal*, 19, 74 (1936).

It is also recommended that the sentence, "Applicable to extracts of anise, lemon, nutmeg, orange, rosemary, thyme, wintergreen, and methyl salicylate" under Essential Oil in Extracts, Method I, be changed to read: "Applicable to extracts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen, and methyl salicylate" and that the following note be inserted in parentheses after the words "top of the column": "Note.—Read from extreme bottom to the bottom of the meniscus at the top of the column for allspice, peppermint, and pimiento extracts."

## REPORT ON MEATS AND MEAT PRODUCTS

By R. H. KERR (U. S. Bureau of Animal Industry,  
Washington, D. C.), *Referee*

The method for the determination of nitrates and nitrites presented at the last meeting by McVey, *This Journal*, 18, 414, 459 (1934), was studied. A set of samples was made up and sent out for collaborative testing. The results were not entirely satisfactory, therefore they will be withheld for the present, and further study of the method undertaken. At the present time the method appears to be reliable or can be made so without any great or far-reaching changes, but the results do not justify any recommendation. Accordingly, the method will be studied further during the coming year.

During the past year a number of inquiries were received requesting information relative to methods for the detection of soybean flour and dried skimmed milk in sausage and other meat food products. Personal experience has also shown that the need for such methods is becoming rather urgent. Accordingly, the following qualitative methods are offered for adoption as tentative methods.

### SOYBEAN FLOUR

*Method I.*—Mix approximately 0.5 gram of the sample with 5 cc. of a solution of urea (20 grams per liter) in a small test tube or flask containing a strip of red litmus paper partially immersed in the liquid. Stopper the tube or flask and hold at 40°C. for 3 hours. Appearance of a blue color in the litmus paper indicates soybean

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 62 (1936).



flour. This test depends on the presence of the enzyme urease which normally occurs in soybean flour. This test is not reliable with products which have been heated to a temperature sufficiently high to destroy the urease.

*Method II.*—Digest 10 grams of the sample in a 150 cc. beaker, covered with a watch-glass, with 50 cc. of alcoholic potash solution (80 grams C. P. stick KOH in one liter of 95% alcohol), with occasional stirring to facilitate digestion.

Transfer to a 100 cc. granulated oil tube (A.S.T.M. form) or other similar pointed tube, washing the sediment from the beaker with a stream of 95% alcohol from a wash bottle. Bring the volume to 100 cc. with 95% alcohol. Mix, and allow the tube to stand for an hour or more, giving it a gentle rotation from time to time to assist sedimentation.

Syphon off the supernatant alcoholic potash solution and examine the sediment microscopically with a magnification of 120–150 diameters. Use a cover slip to cover the preparation. If the sediment is transferred by means of 15–20 cc. of water to a small centrifuge tube and centrifuged, a much better preparation is obtained. Instead of centrifuging, allowing the oil tube to stand after mixing with the water until sedimentation is complete or filtering may be employed.

Look for the large hour-glass or I-shaped cells (sometimes called bearer cells) characteristic of soybeans. Examine with polarized light. The cells stand out quite brilliantly in polarized light. (See Winton, "Microscopy of Vegetable Foods," and Wallis "The Structure of the Soy Bean," British Yearbook of Pharmacy, 1913, pages 467 to 478, for soybean structure.)

A volume of sediment materially exceeding 0.5 cc. (due to spices) in a product containing no starch (except spice starch) warrants suspicion that soybean flour has been used. Identification of characteristic soybean cells in the sediment is proof that soybean flour has been used.

#### DRIED SKIMMED MILK

To 25 grams of the finely divided meat in a 250 cc. beaker add 50 cc. of water. Thoroughly break up the meat with a glass rod and boil the mixture for a few seconds. Filter through a wet filter paper and add 25 cc. of the filtrate to 1 gram of good adsorbent charcoal. Mix by shaking, boil for a few seconds, cool thoroughly, and shake at intervals for 10 minutes. Filter through a small paper or use a filter pump. When the charcoal has completely drained transfer it to a porcelain dish containing 10 cc. of water and 1 cc. of glacial acetic acid. This is best done by opening the paper, holding it by the clean half, and moving it about in the liquid. The greater part of the charcoal is thus removed from the paper. Stir the charcoal with a glass rod and transfer the mixture to a boiling tube. Heat to boiling for about 10 seconds and filter the hot solution through a small paper into a test-tube containing one-half to 1 gram of solid phenyl-hydrazine-hydrochloride and 2 grams of solid sodium acetate. Mix thoroughly and filter from any insoluble oily residue. Place the tube in a boiling water bath and leave it there for 45 minutes. Remove the tube and allow it to stand at room temperature for at least one hour and preferably longer. Pipet off a little of the deposit, if any, and examine it on a slide under the microscope.

Lactosazone crystallizes in characteristic clumps with projecting spines ("hedgehog" crystals). Recrystallize by filtering through a small paper, washing with a small amount of distilled water, and then passing about 4 cc. of boiling water through the paper into a clean tube. Boil the filtrate and pass through the paper two or three times, boiling between every filtration. On allowing the solution to stand, typical crystals of the osazone separate out. Filter, dry, and take the melting point (200°C.).

If the phenyl-hydrazine-hydrochloride solution becomes too concentrated during the boiling process lactosazone crystals may not separate out.

While these methods are only qualitative and not quantitative they should be of material assistance to analysts who are called upon to look for these substances in sausage and other comminuted meat food products. Experience has shown them to be reliable when applied with due regard to their limitations. It is necessary, of course, to remember that soybean flour may be so treated as to destroy urease, and that improved methods of milling might at some future time make it possible to prepare soybean flour that will be free from the characteristic cells, also that addition of pure lactose to sausage would make a product which would yield lactose-osazone. However, when the characteristic soybean cells are found in sausage, addition of soybean flour or soybean product is proved, and the identification of lactose-osazone is conclusive evidence of the presence of lactose. Accordingly it is recommended<sup>1</sup> that the methods be adopted as tentative.

## REPORT ON SPICES

By J. F. CLEVINGER (U. S. Food and Drug Administration,  
New York, N. Y.), *Referee*

A method for the evaluation of ginger based upon results reported in *J. Am. Pharm. Assoc.*, 630-634 (1928), was, in the main, followed in the present collaborative work. The modified method submitted to the collaborators has been published, *This Journal*, 19, 98 (1936).

To determine the practicability of this method, two uniformly coarsely ground samples of African ginger (1 and 2) and one of Jamaica ginger (3) were submitted to O. C. Kenworthy and B. Lubell, New York Station, Food and Drug Administration. The results of assay (expressed in percentage) are shown in the table.

|                                  | Clevenger |       |       | Kenworthy |       |       | Lubell* |       |       |
|----------------------------------|-----------|-------|-------|-----------|-------|-------|---------|-------|-------|
|                                  | (1)       | (2)   | (3)   | (1)       | (2)   | (3)   | (1)     | (2)   | (3)   |
| Resins (%)                       | 5.95      | 5.52  | 3.95  | 5.96      | 5.92  | 3.81  | 6.38    | 6.06  | 4.22  |
| Volatile Oil (cc. per 100 grams) | 2.82      | 2.70  | 2.03  | 2.71      | 2.70  | 1.90  | 1.86    | 2.33  | 1.38  |
| Specific Gravity (25°/25°)       | 0.883     | 0.879 | 0.876 | 0.856     | 0.867 | 0.867 | 0.878   | 0.890 | 0.890 |
| Optical Rotation† (25°/25°)      | -46.0     | -50.1 | -46.7 | -44.8     | -41.0 | -46.4 | -35.8   | -27.0 | —     |
| Refractive Index 20°             | 1.492     | 1.491 | 1.494 | 1.493     | 1.492 | 1.494 | 1.494   | 1.495 | 1.493 |
| Acid No.                         | 2.4       | 2.2   | 4.2   | 1.1       | 2.1   | 2.6   | 3.3     | 6.7   | —     |
| Ester No.                        | 13.0      | 22.0  | 17.0  | 30.2      | 25.5  | 22.0  | 13.2    | 18.0  | —     |

\* Analyses were made 8 months after the ginger was ground and stored in sealed glass jars.

† Angular degrees 25°, 100 mm tube, white light.

Reasonable agreement in the yield of oleoresin (resin and volatile oil) of ginger was obtained. The variations in the yields of resin and volatile oil and the constants for the volatile oils are probably accounted for by

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 63 (1936).

the variation in the elapsed time between grinding of ginger and the assay of the material.

It is recommended<sup>1</sup> that the method presented be adopted as tentative and that further work be done.

## REPORT ON BAKING POWDERS

By G. L. BIDWELL (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The referee gave considerable thought to the methods of this chapter in *Methods of Analysis*. Heidenhain's method for the determination of carbon dioxide is not necessary and should be deleted. It is a good method but Knorr's method is sufficient for referee work, and the Chittick method is almost universally used for routine tests.

The present method, **XV, 15**, does not give the true neutralizing value of monocalcium phosphate, but it is widely used in the trade and gives acceptable results for control work. The following statement should be inserted between the heading and the paragraph: "A method of industrial application useful when approximate results are desired."

B. G. Hartmann devised two methods useful in baking powder analysis. One gives a quantitative measure of the tartrate radical and the other determines the tartaric acid. They are known to give reliable results and should be studied collaboratively. These methods were published in *This Journal*, **13**, 385 (1930).

Because the methods for the determination of lead are out of date, and new methods of much greater accuracy and ease of manipulation are under development, the present methods should be deleted. The same is true of the method for the determination of fluorides, and this method should be deleted.

## RECOMMENDATIONS<sup>2</sup>

It is recommended—

- (1) That the Heidenhain method be deleted.
- (2) That the explanatory subheading suggested in this report be added to paragraph **15**.
- (3) That study of Hartmann's methods for determining tartaric radical and tartaric acid be made.
- (4) That the methods for the determination of lead, **33**, etc., be deleted.
- (5) That the method for the determination of fluorine be deleted.

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, **19**, 65, 98 (1936).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, **19**, 63 (1936).

## REPORT ON NUTS AND NUT PRODUCTS

By S. C. ROWE (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

In last year's report, *This Journal*, 18, 419 (1935), proposed methods of analysis were presented, with the recommendation that they be given collaborative study. Unfortunately, no work was done during the year. However, in view of the fact that these methods have been used successfully in connection with other products or are modifications of methods that have appeared in the literature, it is recommended that the proposed methods be adopted as tentative.<sup>1</sup>

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No report on fish and other marine products was given by the referee.

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## REPORT ON CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The work on cacao products during the past year was limited to the detection of added lecithin and added shell. The Associate Referee on Lecithin, M. L. Offutt, did not submit a report, although some work was done. A number of methods<sup>2</sup> were reviewed, and an attempt was made to select the most suitable one to compare with the method formulated by Winkler and Sale, V(a), published in *This Journal*, 14, 544 (1931). A plan was outlined to investigate the methods with view to incorporating the best steps of all. The work was only partially completed. A preliminary comparison of the two methods<sup>3</sup> that appeared to be the most promising<sup>2</sup> showed better results by the Winkler-Sale method but did not show complete recovery by either method. Investigation should be continued next year.

*Cacao Shell.*—Following out the recommendation of the associate referee last year, further work was done on the crude cellulose method for detection of added shell. The method was subjected to intensive study, resulting in modification of the procedure. Closely agreeing results on four samples containing known amounts of shell were obtained by the writer and a collaborating chemist.

Comparison of the crude cellulose method with the crude fiber method shows a number of advantages in favor of the cellulose method; the

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 62 (1936).

<sup>2</sup> *Z. Untersuch. Lebensm.*, T 61, No. 5, 520-23 (1931); 67, 371-79 (1934); *Bull. Officiel l'Office International Fabricants Chocolat et Cacao*, December, 1932, p. 475, and January, 1934; *Bull. soc. chim. biol.*, May, 1933, pp. 607-18; *This Journal*, 14, 544 (1931).

<sup>3</sup> *Bull. Officiel l'Office International Fabricants Chocolat et Cacao*, January, 1931. Modified. *This Journal* 14, 544 (1931).

spread between nibs and shell is much greater and there is a greater proportional increase in the values obtained on samples containing added shell. It is believed that variation in the determination between varieties of beans will not be so great for the crude cellulose as for the fiber; however, this has not yet been established. The determination does not appear to be affected to any appreciable degree by fineness of grinding, which renders it of greater value.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That methods for the determination of lecithin be further studied.
- (2) That further collaborative work be done on the crude cellulose method for determination of shell.
- (3) That inasmuch as the microscopic method for cacao shell, p. 162, *Methods of Analysis, A.O.A.C.*, 1930, is not used and that work upon it was not completed, the method be deleted (first action).
- (4) That the quantity of sample taken for separation of fat, *Methods of Analysis, A.O.A.C.* 1930, XIX, 16, be changed from "from 2 to 10 grams" to "from 10 to 40 grams" because the amounts given are insufficient for most determinations.

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No report on lecithin was given by the associate referee.

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#### REPORT ON SHELL IN CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

As suggested last year, additional work was done on the cellulose method for the determination of shell. Early in the year a number of samples were sent out to collaborators with a description of the method. Some of the results obtained indicated that the method was not fully understood and that the procedure needed standardization and simplification before check results could be obtained.

After further study and considerable experimentation with various modifications, a revision showing considerable promise was devised, and it was found that reproducible results could be obtained on authentic samples of chocolate liquors containing known amounts of shell. A number of the samples of sweet and bitter liquor were then analyzed by J. B. Wilson and the writer, and good check results were obtained. The re-

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 63 (1936).

sults obtained on four of the authentic samples prepared in a factory under the referee's direction are given in Table 1.

TABLE 1.—*Collaborative results\* on four authentic samples*

| SAMPLE          |                   |  | WILSON          |        | WINKLER         |        | CALCULATED<br>CELLULOSE |
|-----------------|-------------------|--|-----------------|--------|-----------------|--------|-------------------------|
|                 |                   |  | <i>per cent</i> |        | <i>per cent</i> |        | <i>per cent</i>         |
| (1) Bitter      | liquor, 0.95%     |  | 1.51            | (1.60) | 1.45            |        |                         |
| shell           |                   |  |                 |        |                 |        |                         |
| (2) Sweet       | liquor, 5.73%     |  | 2.4             |        | 2.50            | (2.55) | 2.47                    |
| shell           |                   |  |                 |        |                 |        |                         |
| (3) Bitter      | liquor, 10% shell |  | 2.97            |        | 2.90            | (3.0)  | 3.2                     |
| (4) Sweet       | liquor, 14.1%     |  |                 |        |                 |        |                         |
| shell           |                   |  | 3.52            | (3.57) | 3.45            |        | 3.89                    |
| (5) Shell, 100% |                   |  |                 |        | 18.7            |        |                         |

\* On moisture-, fat-, and sugar-free basis. The figures in parentheses are duplicate determinations.

A study of the results reveals a greater spread between shell and nibs than is found in the crude fiber determination, and consistent checks between the analysts. Comparison of the cellulose found with that calculated from the shell content shows very good agreement in the two lower samples, while the amount found in the third is low. However, the results as a whole are satisfactory. The proportional increase on samples containing shell is much greater than that for the crude fiber determination.

It is believed that the method has considerable advantage over the older method when applied to sweet and bitter liquors, as difficulties resulting from the presence of milk solids have not been entirely overcome. The method follows:

#### CRUDE CELLULOSE

(For bitter and sweet chocolate and cocoa)

#### PREPARATION OF MOISTURE-, FAT- AND SUGAR-FREE SAMPLE

Remove the fat, sugar, and moisture from the sample by extracting 10 grams (cocoa), 15 grams (bitter liquor), or 30–35 grams (sweet chocolate) as follows:

Place the roughly weighed sample in a 250 cc. centrifuge bottle and extract twice with 90–100 cc. portions of ethyl ether by shaking vigorously with the reagent, centrifuging, and decanting the supernatant liquor. In a similar manner, extract with 150 cc. and then with 100 cc. of water, centrifuging at 1800–2000 r.p.m. for 10–15 minutes. Decant. Add 60 cc. of alcohol to the residue, stopper, and shake the bottle vigorously. Filter the mixture with gentle suction on a 7 cm. Büchner funnel, using a No. 589 white or blue ribbon filter. Rinse the bottle and wash the residue with 40 cc. of alcohol. When the alcohol just reaches the surface of the cacao residue, add 25 cc. of ether and suck dry.

Transfer the material to a mortar or porcelain dish and pulverize by gentle grinding. Place the powder in an aluminum dish provided with a cover and dry in the oven at 100°C. for 30–45 minutes. Cover the dish when it is removed from the oven and cool in a desiccator.

## REMOVAL OF STARCH

Place two 7 cm. filter papers in each of two aluminum dishes of the same diameter, provided with close-fitting covers. Heat dishes, covers, and filter papers in an oven at 100°–105°C. for at least one hour.

While the dishes and papers are drying in the oven, weigh a 2 gram portion (A) of the dried cacao material and place it in a 500 cc. Erlenmeyer flask. Add 170 cc. of HCl (1+6) in small portions at first and rotate to wet the sample. Heat the mixture to gentle boiling and reflux for 40 minutes. If the determination is run in duplicate, start the heating of the duplicate sample about 5 minutes after the first sample.

After heating as described above, cover the aluminum dishes, cool in a desiccator, and weigh. Remove the filter papers from the dishes, place them in two 7 cm. Büchner funnels, wet them, and adjust vacuum to gentle suction.

Remove the Erlenmeyer flask from the condenser after refluxing 40 minutes and pour the contents, while rotating, onto the Büchner funnel, keeping ready at hand a flask containing 500 cc. of water at room temperature for rinsing the Erlenmeyer flask and washing the residue. CAUTION: Do not permit the residue in the funnel to become dry and crack. To avoid this add the rinse water and wash water portion-wise as the solution in the funnel approaches the surface of the residue. When the last of the wash water reaches the surface of the residue, add 25 cc. of acetone and follow this with another 25 cc. portion of acetone. Turn on the full suction for 5 or 10 minutes. Transfer the residue and paper quantitatively to the same aluminum dish in which they were previously weighed. (By placing the aluminum dish in a larger porcelain dish and inverting the funnel over it the material and filters can be easily transferred by means of a spatula and finally a camel's hair brush.)

Place the aluminum dish and contents in the oven at 100°–105°C. for 1½ hours. Remove from the oven, cover, cool in a desiccator, and weigh accurately. Subtract the weight of the tared dish and filter papers from the weight of tare and contents to obtain the weight of the residue (B).

## EXTRACTION OF CELLULOSE

Weigh a porcelain Gooch crucible, preferably with a removable bottom, prepare in it an asbestos mat, dry, reweigh, and record the weight of asbestos.

Remove the cover from the dish and transfer the contents, including the papers, quantitatively to a 400 cc. beaker. Add 7 cc. of formaldehyde (37%) and mix with a flat-bottomed stirring rod so that all particles are impregnated. Now add 6 cc. of the H<sub>2</sub>SO<sub>4</sub> (1+0.6) and again mix thoroughly with the glass rod. Finally, add 9 cc. of H<sub>2</sub>SO<sub>4</sub> and mix thoroughly. Bring particles on the sides into the liquid with the rod or by rotating the beaker while holding it flat on the desk. Let stand 15 minutes, with occasional stirring, after the addition of the concentrated acid. Add 200 cc. of distilled water and heat the sample to gentle boiling for a minute or two. Remove from the heat and let stand about two minutes for the precipitate to settle. Decant supernatant liquid into the previously prepared Gooch crucible under suction. Wash the residue into the crucible, using a wash bottle, and wash the precipitate with 150 cc. of distilled water. If the crucible runs dry at any time, add a little water and smooth over the cracks with a flattened glass rod. With the addition of 50% alcohol from a wash bottle and a spatula, transfer the residue and asbestos to a tared aluminum dish provided with a cover. Mix the residue with the alcohol uniformly over the dish, using a flat-bottomed glass rod. Evaporate the alcohol on the plate of the steam bath to avoid spattering, then dry in the oven at 100°–105°C.

for 4 or 5 hours or overnight. Cover the dish when removing it from the oven. Cool in a desiccator and weigh.

Obtain the weight of the residue (C) by subtracting the weight of asbestos in the crucible.

Calculate the percentage of crude cellulose in the moisture-, fat-, and sugar-free sample as follows:

$$\frac{B-C}{A} \times 100 = \% \text{ crude cellulose, where}$$

A = weight of moisture-, fat-, and sugar-free sample taken (i.e., 2 grams);

B = weight of residue after removal of starch; and

C = weight of residue after removal of starch and cellulose.

In following the method it is necessary to adhere strictly to the amount of the fat-free, sugar-free sample taken (i.e., 2 grams). A substance like chocolate reacts somewhat to severity of treatment and requires standardization in quantities of sample and of reagents used.

It is recommended<sup>1</sup> that collaborative work be done on the method and that data on various varieties of nibs be obtained.

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No report on coffee and tea was given by the referee.

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No report on gums in foods was given by the referee.

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## REPORT ON OILS, FATS AND WAXES

By GEORGE S. JAMIESON (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.), *Referee*

The collaborative work done on methods of analysis for fats and oils during the past year was under the direction of the associate referees.

T. H. Hopper has no report on the study of the refractometric determination of oil in flax because he was unable to obtain collaborators.

On account of other work the referee was unable to have a collaborative study made on the Kaufmann thiocyanogen method, as was authorized last year.

It is recommended<sup>2</sup>—

(1) That Dr. Lawrence Zeleny, Grain Division, Bureau of Agricultural Economics, be appointed Associate Referee on Refractometric and Other Methods for the Determination of Oil in Flax and Other Seeds.

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 63 (1936).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 61 (1936).



(2) That in *Methods of Analysis*, A.O.A.C., 1930, tentative Method I, under "Detection of Foreign Fats Containing Tristearin in Lard," be eliminated and that Method II be revised as suggested by R. H. Kerr. The revised description of Method II was published in *This Journal*, 19, 97 (1936).

(3) That the method for the detection of coloring matters in food oils that was studied and recommended by Referee C. F. Jablonski be made tentative and inserted under 49, p. 332. This method has also been published, *This Journal*, 19, 95 (1936).

(4) That the specifications for the titer test thermometer submitted by Carl F. Snyder, *This Journal*, 19, 95 (1936), be adopted and substituted for the present ones, 14, p. 317.

(5) That both the Malfatti and the Stout and Schuette procedures be substituted for the present official method, 21(b), p. 321, for the preparation of alcoholic potash. Collaborative study has shown that both of these methods give excellent results.

(6) That further study be made of methods for the determination of acetyl value and hydroxyl number, and that for this purpose Harry Conroy of the North Dakota Regulatory Dept., Bismarck, N. D., be appointed associate referee in place of Willard L. Roberts, who is not in a position at present to direct this collaborative study.

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No report on refractometric determination of oil in seeds was given by the associate referee.

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No report on thiocyanogen value was given by the associate referee.

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## REPORT ON TITER TEST

By CARL F. SNYDER (National Bureau of Standards,  
Washington, D. C.), *Associate Referee*

In *Methods of Analysis*, A.O.A.C., 1930, under Melting Point of Fats and Fatty Acids, 14, p. 317, there is given a specification for the standard thermometer used in the titer test. This specification, which was published in *This Journal*, 2, No. 3, Part II, 302 (1916) is identical in all essential points with the specification given in *Methods of Analysis*, Bulletin No. 107 (Revised). This thermometer was graduated from 10° to 60° C. in 1/10°, and also graduated at 0°. On account of certain of the arbitrary limits in the specification it has always been a difficult and expensive thermometer to manufacture. It appears possible that this thermometer was originally designed to be read to 1/10 or  $\frac{1}{2}$  of a division,

that is, to 0.01 or 0.02, whereas in practice it is read to the nearest division, or perhaps occasionally to  $\frac{1}{2}$  division. It has been difficult to comply with this specification, principally because it was desired to keep the thermometer as short as possible, which resulted in crowding the division marks so close together that reading is not easy.

In view of the difficulties experienced by both manufacturers and users, the Thermometry Section of the National Bureau of Standards prepared a tentative specification for a new titer test thermometer, one which would present no undue difficulties in manufacture and at the same time meet fully the requirements for accuracy in the titer test. This new thermometer is approximately the same length as the original, but it is so designed that the scale, graduated from  $-2^{\circ}$  to  $66^{\circ}$  C. in  $0.2^{\circ}$  C. is sufficiently open to make readings to  $\frac{1}{2}$  division,  $0.1^{\circ}$  C. easy.

In order to obtain opinions on the new specification, copies were sent to practically all the manufacturers of mercury-in-glass thermometers in the United States, as well as to a number of chemists connected with both Government and private laboratories. Two points of interest were brought out in this survey. The titer test thermometer is calibrated for total immersion. It is used, however, for the condition of partial immersion. The opinion was expressed by some that the thermometer should be changed to a partial immersion instrument. The majority of the users, however, feel that since the total immersion thermometer has been used for so long and all published data have been obtained with the total immersion instrument, it would be confusing to make the change. The associate referee concurs in this view, particularly since the titer test is a more or less arbitrary. The difference in reading between the partial and total immersion thermometer would be approximately  $0.4^{\circ}$  C. for the maximum temperature of the instruments.

The suggestion was made by a few of the collaborators that the thermometer be graduated to  $80^{\circ}$  or  $85^{\circ}$ . The large majority, however, expressed the opinion that the range  $0^{\circ}$  to  $65^{\circ}$  C. was entirely satisfactory for this test. One collaborator called attention to the fact that the American Oil Chemists Society has specified two titer test thermometers, one identical with the present A.O.A.C. instrument except that it is graduated to  $65^{\circ}$  C.; the other entirely similar except that it is graduated from  $30^{\circ}$  to  $85^{\circ}$  C. After careful consideration of all the comments and suggestions received from these sources, a specification was prepared, and it appears to meet the requirements.

The specification hereby presented has been written in the standardized form developed in the A.S.T.M. by manufacturers and users, and its provisions conform to accepted manufacturing practice. It has the advantage that the manufacturer will produce the kind of instrument desired without unnecessary cost.

The associate referee wishes to express his appreciation of the as-

sistance given by H. C. Dickinson, E. F. Mueller, and Johanna Busse of the National Bureau of Standards, and by the many manufacturers and chemists.

It is recommended<sup>1</sup> that the specifications submitted for the titer test thermometer be adopted in place of the specifications found in *Methods of Analysis*, 14, p. 317. These specifications were published in *This Journal*, 19, 95 (1936).

## REPORT ON THE HYDROXYL NUMBER AND ACETYL VALUE OF FATS AND OILS

By WILLARD L. ROBERTS (North Dakota Regulatory Department,  
Bismarck, N. Dak.), *Associate Referee*<sup>2</sup>

In accordance with the recommendations accepted by the Association, the collaborative study of the André-Cook,<sup>3</sup> the Roberts-Schuette,<sup>4</sup> and the West-Hoagland-Curtis<sup>5</sup> methods was continued.

Four abnormal samples were submitted to five collaborators. They were prepared by withdrawing four 1200 gram portions from a mixture of 2500 grams of castor oil and 5000 grams of cottonseed oil and treating as follows:

1. *Rancid sample*.—5 grams of rancid cottonseed meal was added to a 1200 gram portion of the stock mixture. This mixture was placed in a shallow pan, over a radiator, in a south window. Additions of water, together with daily vigorous beating with an egg beater, produced a rancid oil in a few weeks. This oil was filtered through an asbestos mat to give a clear brilliant product.

2. *Volatile-soluble acid sample*.—8.0 grams of propionic acid was added to a 1200 gram portion of the stock mixture (0.67% propionic acid).

3. *Volatile-insoluble acid sample*.—21.8 grams of lauric acid was added to a 1200 gram portion of the stock mixture (1.82% lauric acid).

4. *Non-volatile insoluble acid sample*.—31.2 grams of stearic acid was added to a 1200 gram portion of the stock mixture (2.6% stearic acid).

### COLLABORATORS

(1) H. W. Conroy, North Dakota Regulatory Department, Bismarck, N. Dak., C. S. Ladd, Director.

(2) F. Oppen, Department of Chemistry, University of Wisconsin, H. A. Schuette, Director.

(3) Roy W. Riemenschneider, Beltsville Experiment Station, Beltsville, Md.

(4) Albert W. Stout, Linfield College, McMinnville, Oregon.

(5) Edward S. West, Medical School, University of Oregon.

Table 1 gives the results obtained by the André-Cook method.

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 61 (1936).

<sup>2</sup> Present address: Agricultural Chemistry Building, University of Wisconsin, Madison, Wisconsin.

<sup>3</sup> *J. Am. Chem. Soc.*, 44, 392 (1922); *Methods of Analysis*, A.O.A.C., 1930, 326.

<sup>4</sup> *Ind. Eng. Chem. Anal. Ed.*, 4, 257 (1932).

<sup>5</sup> *J. Biol. Chem.*, 3, 104, 627 (1934).

TABLE 1.—*Saponification numbers, acetyl values, and hydroxyl numbers by André-Cook method*

| COLLABORATORS   | SAPONIFICATION NUMBERS |            | ACETYL<br>VALUE | HYDROXYL<br>NUMBER |
|-----------------|------------------------|------------|-----------------|--------------------|
|                 | UNACETYLATED           | ACETYLATED |                 |                    |
| Sample 1        |                        |            |                 |                    |
| Conroy          | 195.7                  | 261.0      | 76.1            | 80.7               |
|                 | 196.7                  | 261.2      |                 |                    |
| Oppen           | 199.1                  | 263.4      | 75.6            | 80.1               |
|                 | 199.2                  | 263.6      |                 |                    |
| Riemenschneider | 200.1                  | 269.2*     | 83.3*           | 88.9*              |
|                 | 200.1                  | 272.6*     |                 |                    |
| Stout           | —                      | —          | —               | —                  |
| West            | 197.5                  | 261.9      | 75.2            | 79.7               |
|                 | 197.0                  | 260.0      |                 |                    |
| Sample 2        |                        |            |                 |                    |
| Conroy          | 194.8                  | 243.9      | 57.5            | 60.1               |
|                 | 194.5                  | 243.6      |                 |                    |
| Oppen           | 196.1                  | 245.1      | 57.6            | 60.2               |
|                 | 196.0                  | 245.4      |                 |                    |
| Riemenschneider | 195.3                  | 248.0*     | 62.4            | 65.5*              |
|                 | 195.5                  | 249.3*     |                 |                    |
| Stout           | —                      | —          | —               | —                  |
| West            | 190.3                  | 239.6      | 57.2            | 59.8               |
|                 | 190.5                  | 237.7      |                 |                    |
| Sample 3        |                        |            |                 |                    |
| Conroy          | 192.9                  | 242.3      | 58.4            | 61.1               |
|                 | 192.0                  | 242.5      |                 |                    |
| Oppen           | 193.8                  | 243.7      | 59.0            | 61.7               |
|                 | 194.3                  | 245.2      |                 |                    |
| Riemenchneider  | 194.2                  | 252.3*     | 66.8*           | 70.3*              |
|                 | 194.0                  | 250.2*     |                 |                    |
| Stout           | —                      | —          | —               | —                  |
| West            | 189.1                  | 241.9      | 60.6            | 63.5               |
|                 | 190.6                  | 241.2      |                 |                    |

TABLE 1.—*Saponification numbers, acetyl values, and hydroxyl numbers by André-Cook method (Continued)*

| COLLABORATORS   | SAPONIFICATION NUMBERS |            | ACETYL<br>VALUE | HYDROXYL<br>NUMBER |
|-----------------|------------------------|------------|-----------------|--------------------|
|                 | UNACETYLATED           | ACETYLATED |                 |                    |
|                 | Sample 4               |            |                 |                    |
| Conroy          | 192.1                  | 242.1      |                 |                    |
|                 | 192.1                  | 242.2      | 58.5            | 61.2               |
| Oppen           | 193.2                  | 245.5      |                 |                    |
|                 | 190.6                  | 244.7      | 62.1            | 65.2               |
| Riemenschneider | 191.4                  | 247.2*     |                 |                    |
|                 | 191.0                  | 247.7*     | 65.4*           | 68.8*              |
| Stout           | —                      | —          | —               | —                  |
| West            | 189.0                  | 242.3      |                 |                    |
|                 | 191.0                  | 242.3      | 60.7            | 63.6               |

\* Not included in the average figures given throughout this paper because apparently they are too high.

Table 2 gives the results obtained by the Roberts-Schuette method.

TABLE 2.—*Hydroxyl numbers by Roberts-Schuette method*

| COLLABORATORS   | HYDROXYL NUMBERS |          |          |          |
|-----------------|------------------|----------|----------|----------|
|                 | SAMPLE 1         | SAMPLE 2 | SAMPLE 3 | SAMPLE 4 |
| Conroy          | 83.4             | 64.0     | 64.5     | 66.2     |
|                 | 83.2             | 64.1     | 64.1     | 65.5     |
| Oppen           | 79.6             | 63.4     | 63.5     | 61.0     |
|                 | 79.5             | 62.2     | 63.7     | 61.9     |
|                 | 78.9             | 65.1     | 65.9     | 61.5     |
| Riemenschneider | 81.5             | 64.9     | 66.9     | 60.4     |
|                 | 81.9             | 65.2     | 66.0     | 65.5     |
|                 | 88.1             | 64.1     | 68.6     | 60.4     |
|                 | 86.5             | 67.2     | 65.3     | 60.9     |
|                 | 79.3             | 65.0     | 68.9     | 65.3     |
|                 |                  | 64.1     | 68.5     | 65.5     |
|                 |                  |          | 71.6     | 63.8     |
|                 |                  |          |          | 65.8     |
|                 |                  |          |          | 66.4     |
| Stout           | 82.2             | 66.0     | 62.5     | 65.1     |
|                 | 82.3             | 64.5     | 62.6     | 65.2     |
| West            | 95.9             | 73.6     | 67.9     | 65.0     |
|                 | 95.5             | 72.4     | 75.4     | 64.8     |

The results obtained by the Roberts-Schuette method (Table 2) show rather good agreement. It should be remembered that each hydroxyl number is obtained by acetylating an individual sample. This is not the case with the André-Cook method, where the hydroxyl number is obtained from the saponification numbers of the original oil and its acetylated product. Except for the results of one collaborator, there is excellent agreement among the collaborators. The results obtained by the André-Cook and the Roberts-Schuette methods agree well except in the cases of Samples 2 and 3, where the added acids were lost in the André-Cook method. The fact that Samples 2, 3, and 4 have nearly identical hydroxyl numbers by the Roberts-Schuette method shows that the blank corrects for the added volatile-soluble and volatile-insoluble fatty acids.

Table 3 gives the results obtained by the West-Hoagland-Curtis method.

TABLE 3.—*Hydroxyl numbers by the West-Hoagland-Curtis method*

| COLLABORATORS   | HYDROXYL NUMBERS |          |          |          |
|-----------------|------------------|----------|----------|----------|
|                 | SAMPLE 1         | SAMPLE 2 | SAMPLE 3 | SAMPLE 4 |
| Conroy          | 63.9             | 51.3     | 52.4     | 50.2     |
|                 | 62.7             | 50.5     | 51.4     | 51.8     |
| Oppen           | 34.7             | 34.2     | 33.6     | 31.7     |
| Riemenschneider | 64.1             | 54.3     | 54.4     | 56.4     |
|                 | 62.6             | 54.3     | 53.2     | 53.7     |
|                 | 63.4             | 50.4     | 52.6     | 51.9     |
|                 |                  |          |          | 54.5     |
| Stout           | —                | —        | —        | —        |
| West            | 70.1             | 57.6     | 57.9     | 56.8     |
|                 | 68.9             | 56.9     | 57.2     | 56.5     |
|                 | 68.7             | 56.8     | 57.4     | 56.2     |

The West-Hoagland-Curtis results (Table 3) show that an individual can obtain satisfactory duplicate results. The results of one collaborator are extremely low and are therefore discarded. Even with the removal of these values very good agreement is not shown among the collaborators. That the blank titration corrects for the volatile-soluble and volatile-insoluble acids is shown by results on Samples 2, 3, and 4, which are nearly identical for each individual, but which vary among collaborators. The results are decidedly lower than those by the André-Cook and the Roberts-Schuette procedures.

Table 4 gives a comparison of average hydroxyl numbers obtained by three methods. Some results in each method, obviously in error, were discarded in arriving at the average values.

TABLE 4.—Comparison of average results, hydroxyl numbers by three methods

| COLLABORATOR    | ANDRÉ-COOK | ROBERTS-SCHUETTE | W-H-C |
|-----------------|------------|------------------|-------|
| <i>Sample 1</i> |            |                  |       |
| Conroy          | 80.7       | 83.3             | 63.3  |
| Oppen           | 80.1       | 79.1             | 34.7* |
| Riemenschneider | 88.9*      | 83.5             | 62.5  |
| Stout           | —          | 82.3             | —     |
| West            | 79.7       | 95.7*            | 69.2  |
| Average         | 80.2       | 82.1             | 65.0  |
| <i>Sample 2</i> |            |                  |       |
| Conroy          | 60.1       | 64.1             | 50.9  |
| Oppen           | 60.2       | 63.3             | 34.2* |
| Riemenschneider | 65.6*      | 65.1             | 53.0  |
| Stout           | —          | 65.2             | —     |
| West            | 59.8       | 73.0*            | 57.1  |
| Average         | 60.0       | 64.4             | 53.7  |
| <i>Sample 3</i> |            |                  |       |
| Conroy          | 61.0       | 64.3             | 51.9  |
| Oppen           | 61.7       | 64.4             | 33.6* |
| Riemenschneider | 70.3*      | 67.8             | 53.4  |
| Stout           | —          | 62.6             | —     |
| West            | 63.5       | 71.6*            | 57.5  |
| Average         | 62.1       | 64.8             | 54.3  |
| <i>Sample 4</i> |            |                  |       |
| Conroy          | 61.2       | 65.9             | 51.0  |
| Oppen           | 65.2       | 61.5             | 31.7* |
| Riemenschneider | 68.8*      | 63.8             | 54.1  |
| Stout           | —          | 65.2             | —     |
| West            | 63.6       | 64.9             | 56.6  |
| Average         | 63.3       | 64.3             | 53.9  |

\* Discarded

## REMARKS BY COLLABORATORS

*Conroy*.—Sample 1, when acetylated, developed a strong color, which tended to interfere with the end point. This was most noticeable in the W.-H.-C. method, where the volume was relatively small. The acetylated product obtained by the W.-H.-C. method apparently is not stable as indicated by the consistently lower results. Considerably more difficulty was experienced in getting determinations to check.

In point of economy of time and reagents the R.-S. method is preferable. After a few trial manipulations, the sample and anhydride may be weighed out and the tube sealed in about 7 minutes.

*Oppen.*—The suggestion is advanced that the R.-S. method be tried on pure organic compounds such as dihydroxy stearin admixed with known inert materials in order that an absolute basis of comparison be established. Results by the André-Cook method are obviously in error with the samples containing volatile-soluble or volatile-insoluble acids.

There is nothing particularly objectionable about the W.-H.-C. method, as regards manipulation, but the accuracy of titrations in butyl alcohol solutions should be investigated. The complexity of the phase relationship made it difficult at times to conduct the titrations. The extremely low results by this method would appear to exclude it from further consideration. Again the need for an absolute basis of comparison is suggested.

*Riemenschneider.*—The same criticisms as were made in 1935 would hold for the 1936 work with an additional note that rancid products and free acidity may possibly affect the conditions for titrating or the actual degree of acetylation of the sample in the R.-S. method. As my results indicate, there was considerable variation in the values obtained for a given sample. Control determinations on Sample C of 1935 were made simultaneously, and excellent agreement with the values for that sample were obtained. It might be expected that the A.-C. method would give high results on samples containing rancid products and free soluble acids. The W.-H.-C. method gave much lower results than either of the other two methods.

*Stout.*—Because of lack of equipment only the R.-S. method was run. A micro buret was used for adding the acetic anhydride.

*West (André-Cook procedure).*—(1) I find the procedure for the preparation of the acetylated sample tedious and time-consuming. (2) The end point is not persistent, the pink color always reappearing after a short time. The recurrence of the color has been associated with a thin gummy layer which formed on the bottom of the flasks in which the saponification was carried out. (3) Loss of soluble acidity in the numerous washings in the preparation of the acetylated sample is greater.

*Roberts-Schuette procedure.*—(1) The general procedure of the method is tedious and laborious. (2) The quantitative transfer of the acetylated sample from the reaction tube by washing with water is difficult. (3) With the exception of "normal non-rancid" samples, the titration of oils in (or on) water is unsatisfactory because a. The end point is not sharp and not permanent, and b. The true value of free acidity is not obtained, as is shown in the table below.

DETERMINATION OF FREE ACIDITY OF OILS

| COLLABORATION SAMPLE NO. | OIL SAMPLE DISSOLVED IN PYRIDINE-BUTYL ALCOHOL (WEST PROCEDURE) | OIL SAMPLE SUSPENDED IN WATER (ROBERTS PROCEDURE) |
|--------------------------|---|---|
|                          | CC. N/2 NaOH (ALCOHOLIC) PER GRAM SAMPLE                        | CC. N/2 NaOH (AQUEOUS) PER GRAM SAMPLE            |
| 1                        | 0.16  | 0.05  |
| 2                        | 0.22  | 0.20  |
| 3                        | 0.22  | 0.11  |
| 4                        | 0.25  | 0.02  |



*West-Hoagland-Curtis procedure.*—Comments on this method are the same as given in last year's report. New modifications of the West procedure have somewhat improved it. This year's results were determined by the modification, and a summary follows:

*Comparison of the methods of Roberts-Schuette, Lewkowitsche-André-Cook, and West-Hoagland when applied to a known mixture of castor oil, oleic acid and butyric acid.\**

| METHOD                  | HYDROXYL NUMBER |                  | THEORETICAL†<br>HYDROXYL NUMBER<br>OF KNOWN MIXTURE |
|-------------------------|-----------------|------------------|---|
|                         | OF CASTOR OIL   | OF KNOWN MIXTURE |   |
| West-Hoagland           | 163.8           | 93.8             | 93.5  |
| Lewkowitsche-André-Cook | 164.9           | 19.1             | 94.1  |
| Roberts-Schuette        | 165.9           | 102.9            | 94.7  |

\* Composition of mixture by weight:

|                  | per cent |
|------------------|----------|
| Castor oil.....  | 57.1     |
| Oleic acid ..... | 28.6     |
| Butyric acid.... | 14.3     |

† The hydroxyl numbers of the castor oil by the different methods are given in Column 2. In calculating the theoretical value of the mixture for a given method, the hydroxyl number of castor oil by the method concerned is used.

## DISCUSSION

The André-Cook method shows poor agreement with normal samples and is inaccurate on samples containing volatile-soluble or volatile-insoluble fatty acids. This method is consuming of both time and materials. It involved the most steps of all the procedures.

The Roberts-Schuette method gives suitable duplicate checks with individuals and individuals check with each other. The blank titration corrects for volatile-soluble and volatile-insoluble fatty acids. It has never been claimed that this blank measures all of the acid present. This is not necessary because the blank is treated in a manner identical with that of the sample during the titration. The only other collaborator to submit blanks run by this method obtained results identical with those obtained by West. This shows the reproducibility of the R.-S. blanks. The associate referee wishes to suggest that the blanks were improperly run by West when the latter tried out the three methods on a mixture of butyric acid, oleic acid, and castor oil.

The R.-S. method appears to be the simplest of the three methods. Fewer steps are involved, fewer reagents are needed, and smaller quantities of materials are used. It is also the most rapid. The associate referee has often completed duplicate runs on a single oil in 2½ hours. During this time a standardization of the alkali in duplicate and of the acetic anhydride in triplicate is carried out.

The West-Hoagland-Curtis method gives suitable duplicate checks with individuals, but the individuals do not check with each other. The

blank titration corrects for volatile-soluble and volatile-insoluble fatty acids.

It is recommended<sup>1</sup> that the Roberts-Schuette and the West-Hoagland-Curtis methods be tried out in a collaborative way on pure organic compounds dissolved in a hydroxyl-free medium such as heavy mineral oil. The compounds recommended for study are heptyl aldehyde, dihydroxy stearic acid, glycerol, and monostearin.

## REPORT ON ALDEHYDE-FREE ALCOHOL

By WILLARD L. ROBERTS (North Dakota Regulatory Department, Bismarck, N. Dak.), *Associate Referee*<sup>2</sup>

In accordance with the recommendations accepted by the Association, a collaborative study was made of the methods of Malfatti<sup>3</sup> and Stout-Schuette.<sup>4</sup>

TABLE 1.—*Preparation of aldehyde-free alcohol by the two methods*

| COLLABORATORS*  | MALFATTI METHOD                         | STOUT-SCHUETTE METHOD                       |
|-----------------|---|---|
| Conroy          | Colorless 5 wks.<br>Colorless 4 mos.    | Faint yellow 5 wks.<br>Strong yellow 4 mos. |
| Oppen           | Colorless 2 days<br>Faint yellow 5 wks. | Faint yellow 2 days<br>Faint yellow 5 wks.  |
| Riemenschneider | Slight straw color 4 mos.               | Colorless 4 mos.                            |
| Roberts         | Colorless 6 wks.                        | Colorless 6 wks.                            |
| Stout           | Colorless 6 wks.                        | Colorless 6 wks.                            |
| West            | Colorless 7 wks.                        | Colorless 7 wks.                            |

\* For addresses of the collaborators, see preceding report.

Table 1 shows that there is little choice between the two methods. Both of them appear more satisfactory than refluxing with potassium hydroxide or treating with silver hydroxide.

### REMARKS BY COLLABORATORS

Conroy.—The method of Malfatti was easier and consumed less time. At the end of four months it also contained less precipitate.

Oppen.—The Stout-Schuette method has been used in the student laboratory for the last three years with very few failures.

Availability of all-glass apparatus must stand in the way of the Stout-Schuette method. The apparatus can be used quite generally for the distillation of other solvents. Given the all-glass apparatus, the Stout-Schuette method is simplest, as grinding the potassium hydroxide and calcium oxide in a mortar is very tedious.

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 61 (1936).

<sup>2</sup> Present address: Agricultural Chemistry Building, University of Wisconsin, Madison, Wis.

<sup>3</sup> C. A., 6, 200 (1912); Vegetable Fats and Oils by George S. Jamieson

<sup>4</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 100 (1933).

Riemenschneider.—The Stout-Schuette method was a little easier than the method of Malfatti, but no significant difference was noted in the time required. The analyst can work on something else while the alcohol is being refluxed or distilled in the Stout-Schuette procedure. The alcohol from the Stout-Schuette preparation had a precipitate settled out after long standing.

Stout.—Our alcohol must have been too good for a suitable comparison.

West.—The method of Malfatti is probably simpler than that of Stout-Schuette because no refluxing or distilling apparatus needs to be set up or watched. Although the latter procedure can be run through in a shorter time than the former, the actual time consumed in the manipulation of apparatus and materials is less in the method of Malfatti.

#### COMMENTS OF THE ASSOCIATE REFEREE

The associate referee has used and has seen used, altogether too often, the methods involving refluxing with potassium hydroxide or destruction of aldehyde with freshly prepared silver hydroxide. These two methods are not satisfactory in many cases.

The methods of Malfatti and Schuette appear on the other hand to be quite satisfactory. The associate referee has used the method of Stout-Schuette many times and has seen it successfully applied by students.

It is recommended<sup>1</sup> that either or both of these two methods be accepted as official.

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No report on microchemical methods was given by the referee.

#### REPORT ON MICROBIOLOGICAL METHODS— CANNED FOODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Because of the delays occasioned by the need for orientation in a pioneer field of activity, there has been conducted, as yet, no organized collaborative work to test certain procedures indicated in the microbiological examination of canned foods. The extensive experience of the several associate referees with applicable methods and culture media has, however, provided the bases for the reports which are being submitted. Progress toward the formulation of approved methods for the microbiological examination of foods in the program outlined previously has in the beginning been slow, but some momentum has now being attained and plans are being made to put to the test some of the procedures and culture media reported on at this meeting.

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<sup>1</sup>For report of Subcommittee C and action of the Association, see *This Journal*, 19, 61, 96 (1936).

Dr. Fellers is making definite recommendations as to procedures for cleansing and disinfecting the container before opening. Dr. Tanner submits some suggestions relative to a method for obtaining material for planting in culture media and the amount of inoculum to be used. Dr. Cameron and Mr. Linden are proposing certain culture media to be used in the bacteriological examination of non-acid foods and acid products, and are suggesting the pH ranges which differentiate these two classes of foods. Dr. James is prepared to make recommendations relative to incubation times and temperatures.

It is quite probable that there will be some dissent to all these recommendations and suggestions. It is further probable that even the several referees will not agree among themselves on some points of procedure. The reports submitted, however, offer a beginning from which point work may be continued.

The plan for the ensuing year contemplates the organization of collaborative workers who, under the supervision of the referees, may attempt to apply, under special test conditions, as well as in routine work, some of the methods and media now suggested. The results of such work should provide a basis for the formulation of a procedure to be recommended at a later date for tentative adoption as an approved method.

In the course of the year's deliberations on the subject of microbiological methods, it has been brought to light that a method for the examination of sugar to determine the presence of microorganisms capable of causing spoilage in canned foods has been in use for several years. This method has been remarkably free from criticism. It is not been considered out of place for the Associate Referee on Culture Media for Non-Acid Foods, who was the originator of the method, to present a description of the procedure for the bacteriological examination of sugar, in connection with the consideration of the assigned topic with which he is particularly concerned. Accordingly, Dr. Cameron has prepared a supplementary report dealing with the bacteriological examination of sugar, which is to be presented for further consideration.

It is recommended<sup>1</sup>—

- (1) That the work on microbiological methods for the examination of canned foods be continued in the light of the plan of collaborative work contemplated by the referee, and be extended to include sugar.
- (2) That the several associate referees who have served during the past year be reappointed and reassigned the same topics studied during the past year.

## REPORT ON TREATMENT OF UNOPENED CONTAINER

By C. R. FELLERS (Massachusetts State College,  
Amherst, Mass.), *Associate Referee*

Containers are of two general types, tin cans and glass jars. While tin cans are of several shapes and sizes, all are hermetically sealed and are treated similarly for bacteriological examination. However, many types of closures are used on glass containers. Most lids are of metal, but a few are of glass. Glass lids present special problems for sampling. The glass jars with metal caps are treated for the most part like tin containers.

1. *Temperature of the container and its contents.*—Since most canned food containers hold a partial vacuum, it is desirable to neutralize this vacuum as much as possible before opening. Otherwise a current of air is sucked into the container. Cans or glass jars should be examined immediately after removal from incubation if possible. If samples are cold they should be allowed to come to laboratory temperature before sampling.

2. *Cleaning the container.*—If very dirty or oily, the container should be scrubbed with a brush and soapy water and dried with a clean towel. If the container is clean to outward appearances, the use of a damp cloth is suggested. The cloth is moistened with a 1 to 1000 solution of bichloride of mercury. It is unnecessary to remove the label unless it is badly soiled.

3. *Sterilizing the top of the container.*—Flaming the top is best accomplished by grasping the container in the hand and holding the previously cleaned top in the flame of a Bunsen burner until there is a positive pressure in the container. This is usually indicated by a snap or flip or by the bulging of the end of the container. The sampling should be accomplished while the container is still hot at the flamed end. If a delay is unavoidable, it is convenient to place one of the parts of a Petri dish over the top of the container.

It is unsafe and inadvisable to flame distended containers or swells. Such containers should be dipped in a 1 to 1000 solution of bichloride of mercury for a few seconds, dried with a sterile towel, and sampled without flaming. To avoid squirting and injury to clothes or laboratory, it is advisable to punch swells with an awl, or can opener well protected by the sterile towel.

4. *Making the opening.*—Two methods are available, depending somewhat upon the character of the canned food and the desirability of removing solid portions of the contents for mass cultures or for any other reason.

(A) The simplest method is to use a previously flamed metal awl. This instrument should be sharp, pointed, and of sufficient length and thickness to make an opening which will admit readily a 10 cc. pipet. The same awl may be cleaned and flamed repeatedly.

(B) Inexpensive, all-metal can openers of simple design are sterilized in quantity and are used as required to open containers. A hole approximately 1.5 inches in diameter is cut out near the center of the top of the container.

If the samples cannot be removed at once after opening the container, a sterile half of a Petri dish should be placed over the top.

## REPORT ON SAMPLING INOCULUM

By F. W. TANNER (University of Illinois, Urbana, Ill.),  
*Associate Referee*

The integrity of results of microbiological examination of canned foods is materially influenced by the type of sample which is used. The difficulties of sampling are quite as apparent with canned foods as with other foods consisting of numerous packets in separate coverings or containers. This problem has been discussed by Esty and Stevenson,<sup>1</sup> but by few others.

### OBSERVATION OF THE UNOPENED CONTAINER

The unopened container should be examined in order to determine whether the ends are flat, and the condition of the seams should be noted. The surface of the can should be carefully examined for evidences of rust. If the ends are concave, as they should be, the presence of vacuum is probably indicated, and likewise the absence of bacterial spoilage.

A. *Examination for Perforations.*—The presence of an undue amount of rust on the can indicates that pin holes and similar perforations may be present. While this condition may be determined with certainty after the can has been emptied, cleaned, and dried, some information may be secured before it is opened by going over it with a stiff, sharp-pointed needle.

B. *Testing for Vacuum.*—If the container has bulged ends, there will, of course, be no need for testing for vacuum. If, however, the ends are concave, some information can be secured by determining the vacuum. The ordinary vacuum gage is provided with a rubber cork, which thoroughly seals the can when the tip of the gage is forced into the cover of the can. The vacuum gage used in the associate referee's laboratory is provided with a side arm carrying a small stopcock into which cotton is forced. After the vacuum has been made, the stopcock may be turned to

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<sup>1</sup> *J. Infectious Diseases*, 36, 486 (1925).

relieve the vacuum. The air that is sucked into the can is filtered through the sterile cotton, thus preventing access of extraneous bacteria.

### OPENING THE PREPARED CONTAINER

A. *Location of Aperture.*—Specimens of food products from the surface layers of the can are undesirable, at least for some products, because these areas, receiving the greatest heat treatment, are less liable to show viable bacteria. Therefore, the aperture through which specimens of food are removed should be so placed that a generous sample may be secured from the center of the can. Heat has penetrated more slowly to this area, and it is here that any possible viable bacteria may be found.

B. *Instruments for Opening Containers.*—Among the various types of instruments used for opening the container are screw drivers, can openers, and a number of other blunt-edged tools. Most of them are objectionable because they may contaminate the product by forcing a piece of metal into the food, and thereby introduce bacteria and disturb the contents of the can. If the can is slack filled, this is not a serious problem, but in some products there is enough free liquor to cause it to flow out over the cover, especially if the can has been heated to sterilize the ends. A disk of metal should be cut from the sterilized cover with a sterile instrument made for the purpose, and the sample should be removed through this aperture. Then a larger portion of the metal may be cut out with the can opener, but the analyst should be very careful to make the opening at least one-half inch from the double seam on the can. The contents of the can may then be carefully removed and the can thoroughly washed and dried to enable further examination of the seams, if that is desired.

C. *Protection of Container After Opening.*—After opening the can, the analyst may protect the top from contamination by placing a sterile cover of a Petri dish over it. The can proper then functions as the lower half of the Petri dish.

### REMOVAL OF SAMPLE

A. *Sample.*—The sample should consist of at least 15 grams of food material. If desirable, a second sample may be stored in a sterile container until the examinations have been completed, and a third sample may be used for enrichment.

B. *Instruments Used.*—The type of instrument used is determined by the sort of food under examination. Liquid food products, such as thin soups or tomato juice, may be sampled with a sterile pipet made from ordinary glass tubing about 14 inches long and having fire-polished ends. With solid portions of food glass tubing is less liable to clog than is the ordinary pipet. Solid food materials, such as sweet potatoes and roast

beef, may be sampled by forcing an instrument made like a cork borer through a one-inch opening in the top of the can and pushing it to the bottom of the can in order to secure as large a sample as possible. In the writer's laboratory special instruments provided with plungers force the sample out quickly into a sterile container, which has been previously prepared to receive it. These instruments are 10 inches long and three-quarters of an inch in diameter.

## REPORT ON CULTURE MEDIA FOR NON-ACID PRODUCTS

By E. J. CAMERON (Research Laboratories, National Canners Association, Washington, D. C.), *Associate Referee*

Reference to the literature relating to the bacteriological examination of canned foods discloses the great diversity of media which have been suggested. This is especially true where the worker's interest has been in other fields and where the canned food study has been temporary and incidental to other lines of research. Under such circumstances, there is an inevitable tendency to adapt to the temporary study on canned foods the individual worker's favorite media, which have possibly been used in fields rather remote from that subject. Where such media have been developed in connection with the study of a single species or limited group of bacteria, they may or may not be of value as a more universal medium. This is illustrated in a striking way by McClung and McCoy,<sup>1</sup> who found that certain media which are of undoubted value in the cultivation of specific anaerobes are distinctly inferior for other anaerobic types.

The media problem has been further complicated and confused by the tendency of some workers to overindulge in the adoption of special media which employ as a base the food which is to be tested. As a general rule, there is no theoretical objection to such procedure, but it introduces the practical disadvantage which comes from the necessity for preparing and keeping in stock a multiplicity of media. Moreover, there is a fallacy in the thought that such media are necessary, because for the different classes of canned foods there are well defined spoilage agents which for growth do not depend upon rare nutrients, such as would be found in one but not in another product. It will of course always be necessary to use special media in order to study specific reactions, but for routine purposes it is believed that they are unnecessary. Fortunately, the present attitude seems generally to be that they are neither necessary nor helpful.

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<sup>1</sup> *J. Bact.*, 28, 267 (1934).



In arriving at a decision regarding appropriate media to be used in testing non-acid canned foods for the presence of dormant or active spoilage bacteria, it is first necessary to define the term "non-acid" in the sense that it is used in the industry and, following that, go over to a consideration of the types of spoilage bacteria most frequently encountered in such products.

The great majority of canned vegetables and other canned products are acid to some degree, but for convenience it has been common to classify them as non-acid, semi-acid, and acid. The non-acid products, such as peas and corn, are in the range of pH 6.0 to 7.0. Semi-acid products, such as string beans, spinach, and asparagus, are commonly between pH 4.5 and 6.0, most of them higher than pH 5.0. The upper limit of the acid range may be taken at about pH 4.5. This is about the point below which spore-forming bacteria cease to be factors in spoilage and it becomes unnecessary to use a process sufficiently severe to destroy them. For the purposes of this report, the so-called non-acid and semi-acid foods will be combined in a single category because similar types of spoilage bacteria are involved.

Spoilage in canned products generally results from understerilization or from leakage of the container after the product has been processed. As would be expected in non-acid vegetables, the bacterial flora differs according to the cause of spoilage. Where spoilage has resulted from leakage, many free-living types may gain entrance and mixed cultures of non-spore forming bacteria are commonly encountered. Usually some of these non-resistant bacteria are gas formers and cause swelling of the container. Spoilage from this cause, however, is a mechanical rather than a biological problem and as such does not call for extended comment here. It is sufficient to say that the media hereinafter to be recommended for non-acid products, while especially adapted to the detection of the organisms causing spoilage from understerilization, are at the same time suitable for the detection of a leakage flora.

Thermophilic bacteria are economically most important as causes of spoilage resulting from understerilization. Three principal spoilage groups have been defined. They have a common characteristic in the production of spores of high resistance to heat. Their optimum growth temperature appears to lie between 50° and 60°C. These three principal thermophilic groups are the "flat sour" thermophiles (*Bacillus stearothermophilus*, Donk), which are facultative anaerobes, and two dissimilar groups of thermophilic anaerobes. One of these anaerobic groups (*Clostridium nigrificans*) is characterized by the production of hydrogen sulfide gas unaccompanied by appreciable proteolytic or saccharolytic changes. The resultant condition in foods is known as "sulfide spoilage." The remaining group is characterized by the production of hydrogen and carbon dioxide

in varying amounts together with production of volatile acids of which butyric acid is predominant. The type species of this group is *Cl. thermosaccharolyticum*, McClung.

Aside from thermophilic bacteria, the remaining group of outstanding importance is the mesophilic group of putrefactive anaerobes, of which the only pathogenic member is *Cl. botulinum* and of which the most resistant member yet found resembles but may not be identical with *Cl. sporogenes*. It is this most resistant type which is of greatest importance from the standpoint of spoilage.

Beyond these groups of demonstrated importance, there are others such as aerobic spore-formers, *Cl. welchii*, and members of the amylobacter group which, by reason of the low resistance of the spores, are infrequently met with. Any general media used in the examination of canned foods should, however, be such as will detect these less important bacteria.

There follows a description of media recommended for use in the examination of canned non-acid foods. These media have been widely used in laboratories interested in canning problems and in general have been found suited to the purpose. In the interests of completeness, the purpose of each medium is indicated and a brief description of cultural manifestations is given.

#### 1. *Dextrose tryptone agar*:

Intended for use in the detection of "flat sour" bacteria.

This medium is prepared as a standardized, dehydrated medium and is marketed under the name of Bacto-dextrose tryptone agar by the Difco Laboratories, Inc., Detroit, Michigan. Because of its standardization, its use in this form is recommended. It may, however, be prepared according to the following formula:

Tryptone, 10 g., dextrose, 5 g., agar, 15 g., bromocresol purple, 0.04 g., and water, 1000 cc.

This medium is used principally for the isolation of flat sour bacteria from original products or from enrichment cultures. It is also suitable for the isolation of other aerobic or facultative anaerobic bacteria, such as may be encountered in non-acid canned foods. For flat sour bacteria, incubation is usually at 55°C. Flat sour colonies are characteristic. The colony is round, measures from 2 to 5 mm. in diameter, presents a typical opaque central spot, and, by reason of acid production in the presence of the indicator, is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant or missing where certain low acid-producing types are concerned or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical sub-surface colonies are rather compact, and may approach the "pin-point" condition.

### 2. Liver broth:

Intended for the detection of thermophilic anaerobes not producing  $H_2S$  (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

In the preparation of this medium, chopped beef liver is mixed with water in the proportion of 500 grams to 1000 cc. This mixture is boiled slowly for one hour, adjusted to approximately pH 7.0, and boiled for an additional 10 minutes, after which the boiled material is pressed through cheese cloth and the liquid is made to 1000 cc. To the broth are added 10 grams of peptone and 1 gram of dipotassium phosphate. The reaction is adjusted to pH 7.0. In tubing, one-half inch to one inch of the previously boiled ground beef liver is introduced into the tube.

Before being used, the medium, unless it is freshly prepared, should be "exhausted" by subjecting to streaming steam for a period of at least 20 minutes. After inoculation, the medium should be stratified with sterile vaseline or preferably with a 2 to 2½ inch layer of plain nutrient agar (common formula), which has been cooled to 50°C. Where the inoculated medium is to be incubated at 55°C., the tube should be preheated in a water bath before it is placed in the incubator.

In this medium, thermophilic anaerobes are evident through the splitting of agar and the presence of acid. At times, a "cheesy" odor is noted. When incubation is at 37°C., the presence of putrefactive anaerobes becomes apparent through splitting of the agar, resulting from gas production, and the presence of a putrid odor.

### 3. Beef heart peptic digest broth:

Intended principally for the detection of putrefactive anaerobes and for their cultural study.

The following description of this medium is taken from the report of Dubovsky and Meyer.<sup>1</sup>

(1) Slowly heat to boiling finely ground, fat-free heart, 1000 g., and tap water, 1000 cc.; adjust to a reaction of pH 8.0–8.2. Then cool and carefully skim off the layer of fat which floats on the cold medium. To each liter of beef heart mash, add 2 liters of peptic digest broth, see (2). Adjust the reaction to pH 7.2–7.4.

(2) Wash clean and mince finely 5 or more large pig stomachs. Mince an equal amount of clean pig or beef liver. Mix in the following proportions: Minced pig stomachs 400 g., minced liver 400 g., hydrochloric acid (Baker Chemical Co.) 40 g., and tap water at 50°C. 4000 g.

Keep the mixture in glass or porcelain receptacles for 18–24 hours. Make biuret and also tryptophan tests. When both reactions are positive, the digest is green-yellowish and contains little undigested debris. Transfer to large bottles and steam for 10 minutes at 100°C. to stop digestion. Strain the digest through cotton or preferably store overnight in the ice chest and decant after 24 hours. Warm the decanted digest to 70°C. and neutralize with sodium carbonate (twice normal solution) to litmus at this temperature. Filter the desired amount, add 0.2% dipotassium

<sup>1</sup> *J. Infectious Diseases*, 31, 505 (1922)

phosphate; adjust to pH 7.4, and mix with beef heart mash. Adjust the final reaction and sterilize for one hour at 18 lbs. of pressure. Incubate for 5 days and repeat the same sterilization for one hour at 18 lbs. of pressure.

Before inoculation, "exhaust" in the manner suggested for the liver broth medium. After inoculation, stratify with sterile vaseline.

For general use, this medium carries the obvious disadvantage that it is difficult to prepare. Where any intensive study of putrefactive spoilage is made, however, it is regarded as a valuable medium.

#### 4. *Corn-liver medium:*

Intended for the detection of thermophilic anaerobes not producing hydrogen sulfide (*Cl. thermosaccharolyticum*), putrefactive anaerobes, and other mesophilic anaerobes.

This promises to be recognized as a most valuable medium for general work requiring the cultivation of anaerobic bacteria. The originators, McClung and McCoy,<sup>1</sup> have subjected it to rigid tests, and it has been shown to be superior to several widely used anaerobic media for the cultivation of a wide range of test organisms. It is an inexpensive medium and its use is highly recommended. For a time, however, it is suggested that it be used in conjunction with another anaerobic medium such as liver broth, in order that comparative data may be obtained. The authors describe its preparation as follows:

The medium is made from ordinary whole corn meal, dried liver, and water. The one or two per cent liver (tissue from liver infusion medium dried at 55° to 60°C. and finely ground) and 5 per cent corn meal are steamed one hour, cooled, and tubed. The resulting, rather viscous, medium requires care in sterilizing; this may be accomplished by autoclaving for 2 hours at 15 to 17 lbs. If pressure is reduced slowly after sterilization, short (6-inch) tubes may be used without blowing the plugs. This medium need not be steamed just before using, requires no seal nor incubation in an anaerobic jar, and satisfactory results may be obtained with 2 to 5 centimeters depth of medium.

Positive cultures are recognized by the appearance of gas with or without digestion of the medium. There may also be a measurable change in the reaction of the medium.

#### 5. *Sulfite agar:*

Intended for the detection of thermophilic anaerobes producing H<sub>2</sub>S (*Cl. nigrificans*).

This medium was developed in the Research Laboratories of the National Canners Association and has been successfully used over a period of several years. Sulfite agar is prepared by adding 0.1 per cent sodium sulfite and 3 per cent sucrose to plain yeast water agar. At the time of tubing, a clean iron strip or nail is placed in the tube. The medium should be used within a week after preparation. Yeast water is prepared by autoclaving at 15 lbs. pressure for 5 hours a 10 per cent suspension of starch-

<sup>1</sup> *Loc. cit.*

free yeast. This is allowed to settle for several days. When clear, the supernatant liquid is decanted and made into a medium containing 1.5 per cent agar. The reaction is adjusted to pH 7.0.

In sulfite agar, the "sulfide" spoilage organisms are detected through the formation of characteristic blackened spherical areas. Due to the solubility of hydrogen sulfide and its fixation by iron, no gas is noted. Certain of the thermophilic anaerobes not producing  $H_2S$  give rise to relatively large amounts of hydrogen, which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.

## REPORT ON METHODS FOR DETECTING AND ESTIMATING NUMBERS OF THERMOPHILIC BACTERIA IN SUGAR

By E. J. CAMERON (Research Laboratories, National Canners Association, Washington, D. C.)

It is appropriate that the report on Culture Media for Non-Acid Products be supplemented by a report on methods for detecting and estimating numbers of thermophilic bacteria in sugar.

Since 1926, the canning industry has been aware that sugar, both beet and cane, may carry spores of all three groups of thermophilic bacteria which are important as spoilage agents in non-acid canned foods, i.e., flat sour bacteria (*Bacillus stearothermophilus*), thermophilic anaerobes, not producing hydrogen sulfide (*Clostridium thermosaccharolyticum*), and sulfide spoilage bacteria (*Cl. nigrificans*). For a somewhat shorter period, the sugar industry has been aware of this condition and has taken steps to control thermophilic contamination in its products. Since these bacteria are economically the most important causes of spoilage through under-sterilization of non-acid canned vegetables and since all general methods for the examination of such products must include a routine search for thermophilic bacteria, the culture media described in the Report on Culture Media for Non-Acid Products, apply as well in the examination of sugar.

The methods for sugar hereinafter to be described were given publicity in 1931 as part of a statement on Bacterial Standards for Sugar, set up by the National Canners Association as the basis for its judgment regarding the suitability of sugar to be used in canning non-acid products. Since that time these methods have enjoyed wide use by control laboratories connected with the canning industry, by the sugar industry, by government and state laboratories, and by university departments interested

in this problem. During this period no criticism of the method has been published nor has any been received by the National Canners Association in correspondence. There has been a limited revision of the method since 1931. A description of existing procedure is as follows:

## METHODS

### SAMPLING

Take one-half pound samples from each of 5 bags or barrels of the shipment or of the lot in question. Send these samples to the laboratory in clean sealed cans, or other appropriate containers.

It is appreciated that the adequacy of this sampling will vary in relation to the size of the shipment or lot, but it is felt that where there is any significant variability in the shipment, this fact will become evident, in the majority of cases, through individual tests on five samples.

### PREPARATION OF SAMPLE

Place 20 grams of sugar in a sterile 150 cc. Erlenmeyer flask marked to indicate a volume of 100 cc. Add sterile water to the 100 cc. mark. Bring rapidly to boiling, and boil for 5 minutes. Replace evaporation with sterile water.

### DETECTION OF FLAT SOUR SPORES

Into each of 5 Petri dishes pipet 2 cc. of the boiled sugar solution. Cover and mix the inoculum with dextrose tryptone agar (described in the Report on Media for Non-Acid Products, p. 435). Incubate the plates at 55°C. for 36-48 hours. In order to prevent drying of the agar, the incubator should be humidified. The combined count from the 5 plates represents the number of spores in 2 grams of the original sugar. Multiply this count by five in order to express results in terms of number of spores per 10 grams of sugar.

Flat sour colonies are characteristic. The colony is round, measures from 2 to 5 mm. in diameter, presents a typical opaque central "spot," and by reason of acid production in the presence of the indicator, is usually surrounded by a yellow halo in a field of purple. This halo may be insignificant, or missing, where certain low acid-producing types are concerned, or where the plate is so thickly seeded that the entire plate takes on a yellow tinge. The typical sub-surface colonies are rather compact and may approach the "pin point" condition.

Where there is doubt as to the identity of the sub-surface colonies, a decision can usually be made by observing the nature of the surface colonies. If they evidence reasonable purity of flora, it is safe for practical purposes to assume that the sub-surface colonies have been formed by similar bacterial groups. It is emphasized that where the plate is heavily seeded, there may be loss of accuracy as regards counts, and colony structure and size may be atypical. Where plates are so heavily seeded as to make counting impracticable, a second sample of the sugar may be plated, dilutions of the original solution being used.

At times, the nature of sub-surface colonies may be in question. Whether they are "flat sour" colonies may often be determined by trans-

ferring, by the stroke method, from the colonies to agar plates. Their surface characteristics may then be noted. No immediate significance is attached to the presence of "non-spoilage" thermophiles; i.e., aerobic spore-formers, actinomycetes, etc.

#### DETECTION OF THERMOPHILIC ANAEROBES NOT PRODUCING $H_2S$

Divide 20 cc. of the sugar solution approximately equally among 6 liver broth tubes, as described in the Report on Media for Non-Acid Products, p. 436, and stratify the liquid medium with plain nutrient or yeast water agar. After the agar has solidified, preheat to 55°C. and incubate at that temperature for 72 hours.

Under the conditions stated, thermophilic anaerobes are manifest through the splitting of agar and the presence of acid. At times a "cheesy" odor is noted. The method is considered suitable as a qualitative test but quantitatively it provides only a means for estimation. The method does not permit expression of results in terms of numbers of spores per unit weight of sugar.

#### DETECTION OF THERMOPHILIC ANAEROBES PRODUCING $H_2S$ (SULFIDE SPOILAGE ORGANISMS)

Divide 20 cc. of the sugar solution approximately equally among 6 tubes containing sulfite agar, prepared as described in the Report on Media for Non-Acid Products, p. 437. Make inoculations in freshly exhausted deep agar tubes. Incubate at 55°C. for 72 hours.

In sulfite agar the sulfide spoilage organisms are detected through the formation of characteristic blackened spherical areas. Due to the solubility of hydrogen sulfide and its fixation by the iron, no gas is noted. Certain of the thermophilic anaerobes (not producing  $H_2S$ ), methods for the detection of which precede, give rise to relatively large amounts of hydrogen, which splits the agar and reduces the sulfite, thereby causing general blackening of the medium. This condition, however, is readily distinguishable from the restricted blackened areas mentioned previously. The blackened areas may be counted to obtain quantitative results.

#### REPORTING RESULTS

Report flat sour and sulfide spoilage results as number of spores per 10 grams of sugar. Report thermophilic anaerobes (not producing  $H_2S$ ) as number of tubes positive and number negative in the following manner: +++ , --- .

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### REPORT ON CULTURE MEDIA FOR ACID PRODUCTS

By B. A. LINDEN (Bacteriological Section, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In the field of food bacteriology, innumerable media have been devised for the bacteriological examination of non-acid canned foods. In addition,

workers in this field have devised modifications of standard well-known media, which they have adapted to the isolation of specific organisms associated with various types of spoilage in these products. Culture media adapted to the detection of spoilage in acid canned foods have not been developed so extensively and have commonly been limited to media for special products. Some of the media employed in the examination of non-acid and semi-acid foods have found a limited application in the examination of acid canned foods. As the growth requirements of certain groups of microorganisms responsible for spoilage in acid foods differ in many instances from those commonly encountered in spoiled non-acid foods, special culture media are required for their isolation.

Before the types of culture media indicated for the bacteriological examination of acid canned foods are considered, the term "acid foods" should be defined. While nearly all canned foods are acid to some extent, the term is applied to foods having a hydrogen-ion concentration that will inhibit the growth of most of the microorganisms commonly encountered in non-acid canned foods. The pH range of this class of foods varies between 4.5 and 2.0, and there are occasional instances of overlapping at the upper pH limits into the semi-acid class of foods. Spore-bearing bacteria do not commonly produce spoilage in acid foods, except in the upper limits of the pH range. Thermophilic bacteria are occasional exceptions.

The types of acid canned foods can briefly be classified into five general groups: 1. Tomato Products, 2. Fruits and Fruit Products, 3. Fermented Foods, 4. Marinated Foods, and 5. Beverages.

There are two objectives sought in the bacteriological examination of acid canned foods, the main one being the detection of microorganisms responsible for spoilage, and the minor the detection of dormant microorganisms. The latter may be attenuated, or inhibited, and capable of producing delayed spoilage under suitable conditions. Other factors include the types of spoilage commonly encountered in these products, and the groups of microorganisms associated with them. There are three common types of spoilage of microbiological origin in acid foods: bacterial, yeast, and flat sour. A fourth type of abnormality, hydrogen swells, results from attack on the metal of the can by the acid in the food. Hydrogen swells are usually sterile.

In bacterial spoilage of acid canned foods, tomato products are perhaps of greatest economic importance. Fruits and fruit products, and fermented foods, such as sauerkraut and cucumber pickles, are also subject to bacterial spoilage. The organisms ordinarily responsible for bacterial spoilage in this class of foods are acid tolerant and frequently are capable of producing gas, resulting in hard swells. An organism, *Lactobacillus lycopersici*, was isolated from spoiled ketchup and described by Mickel



and Breed.<sup>1</sup> The same organism was observed by Esty,<sup>2</sup> and it was also studied by Pederson,<sup>3</sup> who described five other species of bacteria belonging to the same genus held to be the responsible agents in the spoilage of tomato products. Members of this group of acid-tolerant organisms have been isolated by the associate referee from other spoiled acid canned foods, such as chili peppers in wine sauce, artichokes in olive oil and acid sauce, Worcestershire sauce, sauerkraut, sour beer, pickled beets, and cucumber pickles. A closely related organism associated with spoilage of tomato products, *Leuconostoc pleofructi*, was originally isolated from spoiled fruit juices by Savage and Hunwicke.<sup>4</sup> Acetic acid bacteria and micrococci are associated with spoilage of marinated products, which may also carry lactobacilli.

Spoilage by yeasts has caused considerable economic loss in practically all acid food products. Fruits and fruit products, tomato products, and beverages are the main classes affected. The yeasts are also aciduric organisms and are encountered in spoilage of acid foods comparatively high in carbohydrates. In tomato products the carbohydrate is present as invert sugar.

Flat sour spoilage is less frequently encountered in acid canned foods than in non-acid foods. It commonly is caused by underprocessing and slow cooling, permitting development of thermophilic bacteria. These organisms form acid, but no gas, from carbohydrates. They produce heat-resistant spores and grow best between 50° and 60°C. An example of this type of spoilage is flat-sour tomato juice.

No universal culture medium has been devised for the detection of all groups of organisms involved in acid food spoilage. While certain special media are of value in specific instances where strict growth requirements must be met, it is neither desirable nor necessary to resort to a multiplicity of media containing as their basic ingredient some of the food product under examination. In the preparation of culture media for the detection of spoilage bacteria in acid products, consideration must be given to the growth requirements of the non-sporulating mesophilic, facultative, anaerobic, aciduric lactobacilli. Other lactobacilli are not so exacting in their growth relationship to oxygen, and aciduric cocci are perhaps the least exacting in this regard. The growth requirements of the yeasts demand oxygen, carbohydrates, and an acid environment.

A study has been started on various types of culture media for the isolation of spoilage organisms of the type *Lactobacillus lycopersici*. Based on previous work in the development of suitable media for the isolation of *Lactobacillus acidophilus*, a series of experimental media was tested on a practical basis to ascertain which media and technic were most satis-

<sup>1</sup> New York State Agr. Expt. Sta. (Geneva) Tech. Bull. 110.

<sup>2</sup> Canner, 60, 94 (1925).

<sup>3</sup> New York State Agr. Expt. Sta. (Geneva) Tech. Bull. 150.

<sup>4</sup> Special Rpt. No. 16, Food Investigation Board, London (1923).

factory in detecting spoilage organisms in canned tomatoes, canned chili peppers, fermenting Worcestershire sauce, and similar products. A search of the literature revealed that similar investigations on culture media had been conducted by Mickle and Breed<sup>1</sup> and Pederson.<sup>2</sup>

Although the media given have been found most useful, additional investigation is necessary to determine which medium is most satisfactory. It may be noted here that gas production by the bacteria involved in tomato products spoilage is absent, or limited, depending upon the degree of anaerobiosis obtained in the medium during incubation.

### 1. *Tomato-Juice Broth (Mickle & Breed) and Tomato-Pulp Broth*

|                                 | cc. |
|---------------------------------|-----|
| Nutrient broth                  | 900 |
| Tomato juice,<br>or tomato pulp | 100 |
| Reaction pH 6.8-7.2             |     |

Place the material in Durham fermentation tubes. Sterilize under 10 lbs. pressure for 15 minutes, or fractionally in an Arnold sterilizer for 30 minutes on 3 successive days. Incubate between 20° and 41°C. (Room temperature is satisfactory.)

### 2. *Bacto Tomato Juice Agar (Dehydrated)*

(Kulp and White Modification)

This medium is satisfactory for a plating medium.

|                                  | grams |
|----------------------------------|-------|
| Tomato juice (400 cc.)           | 20    |
| Bacto peptone                    | 10    |
| Bacto peptonized milk            | 10    |
| Bacto agar                       | 12-15 |
| Distilled water to make 1000 cc. |       |

Pour plates fairly thick to provide for sub-surface growth.

### 3. *Pederson's Yeast Water-Tomato Juice*

|                       | cc. |
|-----------------------|-----|
| Yeast water           | 900 |
| Filtered tomato juice | 100 |
| Reaction pH 6.6-6.8.  |     |

Autoclave at 15 lbs. pressure for 5 hours a 10 per cent suspension of starch-free yeast. Allow to settle in the refrigerator several days. When clear, decant the supernatant liquid.

Place in Durham fermentation tubes and sterilize in the autoclave at 10 lbs. pressure for 15 minutes, or fractionally in an Arnold sterilizer. For a plating medium dissolve 1.5 per cent agar in the yeast water and add the tomato juice just before sterilization.

<sup>1</sup> *Loc. cit.*

<sup>2</sup> New York State Agr. Expt. Sta. Bulls. 150 and 151.

4 *Bacto Trypsin Digest Agar (Cheplin)*

(Uniform composition and convenient form)

|                            | grams |
|----------------------------|-------|
| Trypsinized milk (800 cc.) | 20    |
| Tomato juice (200 cc.)     | 10    |
| Bacto peptone              | 8     |
| Dextrose, C.P.             | 4     |
| Dextrin, C.P.              | 4     |
| Bacto agar                 | 13-15 |
| Final pH 6.1-6.2           |       |

Dissolve 59 grams of the powder in 1000 cc. of distilled water by boiling, or in the Arnold sterilizer. Distribute into suitable containers and sterilize in the autoclave at 15 lbs. pressure for 15-20 minutes.

Twelve experimental media have been prepared and tested on a limited number of products. For the sake of brevity, the medium which gave the most satisfactory results will be described.

*Digest-Yeast-Tomato Juice (Linden)*

|                         |          |
|-------------------------|----------|
| Tryptic milk digest     | 100 cc.  |
| Bacto yeast extract     | 2 grams  |
| Tomato juice            | 100 cc.  |
| Dextrose                | 10 grams |
| Distilled water to make | 1000 cc. |

Prepare the tryptic milk digest as follows: To a liter of skim milk, add sufficient anhydrous sodium carbonate to adjust the reaction to pH 8.0. Add a paste consisting of 2 grams of Fairchild's trypsin, or 2 cc. of freshly prepared enzyme. Add 25 cc. of chloroform and incubate at 30°C. for 24-48 hours. Agitate at intervals and maintain the optimum pH. After digestion neutralize with 10 per cent hydrochloric acid, and heat in a double boiler or on a water bath to remove the chloroform. Sterilize 100 cc. aliquots in the autoclave for 15 minutes at 15 lbs. pressure.

Place in fermentation tubes, or medium sized test tubes, and sterilize in the autoclave for 15 minutes at 15 lbs. pressure, or fractionally. Before using, heat the tubed medium in boiling water 10 minutes, cool, and inoculate. When fermentation tubes are not available, overlay the tubed broth with vaspar seal. For a plating medium, add 15 grams of agar and dissolve by boiling, or heating, in an Arnold sterilizer, adding the tomato juice before sterilization.

The addition of 10 per cent tomato juice to Holman's Cooked Meat Medium<sup>1</sup> gave satisfactory results for the isolation of the aciduric bacterial flora of acid canned foods, undergoing decomposition. It is desirable to note also that a partial atmosphere of carbon dioxide in many instances appears to enhance the growth of the lactobacilli on solid culture media. While some of the culture media given above may serve to detect the presence of yeasts, the special media commonly used are acid in reaction and contain suitable carbohydrates.

<sup>1</sup> *J. Bact.*, 4, 149 (1919).

The medium most extensively used is the Beer Wort, or Malt Extract Medium, suggested by Eyre.<sup>1</sup> Various modifications exhibit the same difficulty with this medium, that is, formation of precipitates during sterilization. Recently Hall and Lothrop<sup>2</sup> devised a satisfactory yeast medium, utilizing the clarifying principle of a 5 per cent suspension of Bentonite on honey. This clarification technic has been applied to malt extract media with favorable results. The directions follow:

*Clarified Malt Extract Medium for Yeasts and Molds*

|                          |           |
|--------------------------|-----------|
| Dry malt extract (Difco) | 100 grams |
| Distilled water          | 1000 cc.  |

Dissolve the powdered malt extract in the water by heating in an Arnold sterilizer, or on the water bath. Adjust to pH 4.7 and cool to 50°C. Add slowly 100 cc. of a 5% suspension of Bentonite (colloidal clay) and mix vigorously. Hold at 50°-75°C. for 30 minutes, then filter through a fluted paper filter until clear. Heat the filtrate in the autoclave 10 minutes at 15 lbs. pressure, and filter through paper to remove any precipitate formed. Distribute into tubes, or flasks. For a plating medium, dissolve by heating, 2% agar-agar in the clarified broth, and filter if necessary through cotton and cheese cloth. To avoid further precipitation sterilize at 10 lbs. pressure for 15 minutes and cool promptly.

*Media for the Detection of "Flat-Sour" Organisms*

The bromocresol purple dextrose tryptone medium, recommended by Cameron in his Report on Culture Media for Non-Acid Products, p. 433, has been found useful in the detection of thermophilic bacteria occurring in acid canned foods.

## REPORT ON INCUBATION PERIODS AND TEMPERATURES FOR CULTURES

By L. H. JAMES (U. S. Bureau of Chemistry and Soils,  
Washington, D. C.), *Associate Referee*

In selecting the proper times and temperatures for incubation of cultures prepared from canned products, careful consideration must be given to the type of spoilage commonly encountered with the food product under examination. It is important also that the purpose of the examination be thoroughly understood, whether it be to determine the sterility of an apparently normal product or the cause of spoilage. In the former case, there may be but few living bacteria present, whereas in spoiled products the reverse would be true.

The condition of the container may be an indication of the types of microorganisms to expect. When the examination is a test of sterility, it is important that the unopened container be incubated before examina-

<sup>1</sup> *Elements of Bacteria Technique*. Saunders, Philadelphia (1903).

<sup>2</sup> *J. Bact.*, 27, 349 (1934).

tion, because the few living cells may be omitted in the sampling, and because as a rule the analyst is not primarily interested in the actual numbers of the living bacteria present. There are, of course, exceptions, as when studies of heat penetration and efficiency of sterilization are being made. The normal container should be incubated to promote the growth of mesophilic, as well as thermophilic, organisms, so that a representative number of containers should be incubated at temperatures suitable for both types.

The swollen container need not be incubated prior to examination because the number of living organisms present is probably large and also because the container may burst.

A correlation or preferably a standardization of the times and temperatures for incubation is of paramount importance. Surveys have shown that different laboratories use widely different temperatures and periods for the incubation of cultures from canned foods. This is unfortunate because it prevents a proper evaluation of the results from different laboratories.

On the basis of their temperature relationships, the microorganisms significant in canned foods spoilage may be divided into two classes: thermophiles or those growing at high temperatures, and mesophiles or those growing at lower temperatures. Instances are recorded in which the cultivation of thermophiles from canned foods has been attempted at temperatures ranging from 45° to 70°C. There is no need, in fact there is decided objection to the use of temperatures over such a wide range.

The incubation of subcultures for thermophiles can also be too low; first, because of the growth obtained not all microorganisms will be true thermophiles; second, because in all probability, some of the organisms appearing on the plates will not be "spoilage" thermophiles, thus markedly confusing the results obtained; and third, because in many incubators the fluctuations in temperature, as well as the temperatures found at different points in an incubator at any one time, may cover a surprisingly wide range. Thus, in incubators presumably adjusted to 45°C., it is entirely possible at certain times to have the temperature of some areas fall as low as 38°-40°C. This results in the growth of microorganisms other than true thermophiles and obviously confuses the interpretation of the results.

There are also objections to the incubation of subcultures at too high a temperature; first, because only a relatively small percentage of the thermophiles will develop (at temperatures of 65°-70°C. certain types may be entirely suppressed); second, the growth of microorganisms in the upper range of thermophilic life is slow, necessitating long incubation of the cultures with the inevitable drying of the culture media, leading to doubtful results.

The incubation of subcultures from canned foods at temperatures of 55°C., or within a range of 52°–58°C., appears to be the most practical for general use.

The mesophilic group of microorganisms often sought in canned foods grows generally at warm temperatures, 30°–42° C. For these bacteria there are also objections to incubation at too high a temperature; first, because usually only those microorganisms capable of growth at temperatures slightly above that of the human body are obtained; second, incubation at 40°–42°C. will eliminate the growth of many types arising from soil or raw material contamination; and third, because the bacterial count obtained at this temperature would be of doubtful value.

Too low an incubation temperature for these types of microorganisms is also objectionable because it may eliminate those organisms which would grow best at body temperature and also because of the long incubation period necessary.

In a selection of the most suitable temperatures for the incubation of subcultures for this second group of microorganisms, there are many factors to be considered. The bacteria in this group originate largely from the soil or from factory equipment, and it is seldom necessary to consider microorganisms that have gained entrance to the container through contamination from the human body. Thus, the question might logically be raised: Is there any real value in the incubation of subcultures at the temperature of 37.5°C.? Recent studies of methods used in the examination of food products have shown that temperatures of 32°–35°C. may give a truer picture of microbial content. Incubation at the temperature of 37.5°C. generally leads to rapid growth by those types capable of development, but it may be sufficiently restrictive to delay the growth of soil types which are more accustomed to temperatures of 30°–32°C.

At times the incubation of culture plates at the temperature of the human body has been understood as an attempt to isolate bacteria arising from fecal contamination. While obviously erroneous, nevertheless this idea has produced a misconception of the purposes for the analyses of canned foods. The selection of a temperature for the incubation of subcultures intended for the growth of the mesophilic types of microorganisms should receive further study.

In the examination of drinking water and other products it is customary to include subcultures in gelatin media in order to bring out those types of bacteria possessing gelatinolytic properties and capable of growth at still lower temperatures. It is doubtful whether such microorganisms are of any significance in canned foods except in rare instances.

Perhaps it should also be pointed out that not infrequently subcultures from both normal and swollen containers may yield entirely negative results, and that this is not always due to the use of the improper tem-

perature or culture media. When spoilage occurs in certain types of food products in which marked acidity is developed, all the living bacteria may be killed and the subcultures indicate a sterile condition. When the results of the subcultures are negative and the test material shows other definite signs of spoilage a microscopic examination of the product will show the presence of large numbers of dead bacterial cells.

During the coming year, certain phases of the problem of selecting the most suitable temperatures and incubation periods for the microbiological examination of canned foods should receive first attention. The selection of the proper temperature for the incubation of cultures for determining "total count" is of paramount importance. At the same time studies should be made of the length of the period of incubation most suitable, particularly for the microorganisms growing at warm temperatures. Information on the subculture of thermophiles is fairly well worked out, although it should receive further tests and study in the development of recommended methods.

#### ASSOCIATE REFEREES

The following associate referees have been appointed since the list was published in *This Journal*, 19, 3 (1936):

*Cubeb*: J. F. Clevenger, U. S. Food and Drug Administration, New York City.

*Methods for the Titration of Alkaloids*: R. L. Herd, U. S. Food and Drug Administration, Buffalo, N. Y.

*Nitroglycerin in Mixtures*: O. A. Kenworthy, U. S. Food and Drug Administration, New York City.

*Carbon Dioxide in Self-Rising Flour*: Howard Adler, 141 W. Jackson Blvd., Chicago, Ill.

## CONTRIBUTED PAPERS

### FACTORS AFFECTING THE DETERMINATION OF AVAILABLE PHOSPHORUS IN CALCINED PHOS- PHATE AND OTHER WATER-INSOLUBLE PHOSPHATES\*

By K. D. JACOB, L. F. RADER, JR., and T. H. TREMEARNE  
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Calcined phosphate, a material prepared by heating phosphate rock at high temperatures in the presence of water vapor (28, 34, 39, 40)<sup>1</sup> has been made experimentally in this country for several years, and it seems likely that commercial production will be achieved at an early date. The properly prepared material is only slightly soluble in water, but is very soluble in neutral ammonium citrate and 2 per cent citric acid solutions.

In several series of pot tests, Brown, Reid, and Jacob (8), and Jacob and co-workers (24) have shown that the nutrient value of citrate-soluble calcined phosphate is at least equal to that of an equivalent quantity of superphosphate. The nutrient value of calcined phosphate seems to be more closely correlated with the solubility of the phosphorus in 2 per cent citric acid than in neutral ammonium citrate (24). Likewise, the solubility in 2 per cent citric acid seems to be a better index of the nutrient value of steamed bone meal, basic slag, ground phosphate rock, and synthetic calcium phosphates (at least under certain conditions of plant growth) than is the solubility in neutral ammonium citrate (5, 29). On the other hand, Bartholomew and Jacob (5) show that the citrate solubility gives, in general, the better indication of the nutrient value of iron and aluminum phosphates. Jacob, Rader, and Ross (27) observe that the water-insoluble phosphorus in superphosphate is usually much more soluble in neutral ammonium citrate than in 2 per cent citric acid; they attribute this difference in solubility to the presence of iron and aluminum phosphates, either or both.

The official methods of analysis (37) specify the citric acid method for determining available phosphorus in basic slag, whereas all other phosphate fertilizers are evaluated by the neutral ammonium citrate method. Inasmuch as calcined phosphate resembles basic slag in many respects, it seemed desirable to study the factors affecting the solubilities of these and other phosphatic materials in citric acid and ammonium citrate solutions. The results, which are given in this paper, should aid in the selection of the method best adapted to the determination of the available phosphorus in calcined phosphate.

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\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November, 1935.

<sup>1</sup> *Italic numerals in parentheses refer to "Literature Cited" at the end of the paper*



## MATERIALS AND ANALYTICAL METHODS

The samples of calcined phosphate were prepared experimentally by heating Tennessee brown-rock phosphate at about 1400°C. in the presence of water vapor (28). The basic slags were imported materials. The samples of Rhenania phosphate were taken from commercial material that was manufactured in Germany by heating phosphate rock with alkali salts, as described by Messerschmitt (35) and Riefler (41). The Non-acid phosphate was manufactured commercially in 1925 by the Non-acid Fertilizer and Chemical Company, Lakeland, Florida, by fusing a mixture of phosphate rock and potassium sulfate or carbonate in a direct oil-fired rotary kiln (2); the company ceased operation several years ago.

The dicalcium phosphate was a commercial fertilizer material, prepared by neutralizing a hydrochloric acid solution of Florida pebble phosphate with lime. Precipitated tricalcium phosphate No. 287 was a C.P. material sold by Eimer and Amend. Precipitated tricalcium phosphate No. 1197 was prepared by carefully ammoniating a slurry of C.P. monocalcium phosphate and gypsum, as described by Ross, Jacob, and Beeson (49). The  $\beta$ -tricalcium phosphate was prepared by neutralizing an aqueous suspension of pure calcium hydroxide with the theoretical quantity of a dilute solution of pure phosphoric acid, evaporating to dryness on the steam bath, and igniting to constant weight at 900°C. The  $\alpha$ -tricalcium phosphate was prepared by heating  $\beta$ -tricalcium phosphate at 1400°C. The reversible transformation of  $\beta$ - into  $\alpha$ -tricalcium phosphate occurs at approximately 1200°C. (7, 32, 51, 54). The hydroxyapatite was prepared in the manner described by Marshall and co-workers (34).

The samples of bone meal, phosphate rock, and superphosphate were taken from commercial materials that have been or are now being produced in this country.

Except as it is stated otherwise in the tables and the accompanying text, the determinations of ammonium citrate- and citric acid-insoluble phosphorus were made as follows:

With the exception of the superphosphates, which were put through a 20-mesh sieve, the samples were ground to pass either an 80- or a 100-mesh sieve.

Citrate-insoluble phosphorus was determined by the official method (33, 37), which involves the digestion of a 1 gram sample with 100 cc. of neutral ammonium citrate solution for 1 hour at 65°C. The preliminary washing with water to remove water-soluble material was omitted; the extracts were filtered by suction through a double thickness of Whatman's No. 5 paper; and the residues were washed with water at the temperature of the citrate digestion. In the digestions with filter paper the sample and one 9 cm. paper were added at the same time to the citrate solution. Complete or practically complete pulping of the paper was usually obtained at the first shaking of the flask.

The general procedure for the extraction of the sample with 2 per cent citric acid was the same as that outlined in the official method for available phosphorus in basic slag (37). The flask was shaken in an end-over-end shaker at 18 r.p.m. In order to conserve the materials, the digestions were made on 2 gram samples with the proportionate quantities of alcohol and citric acid. The insoluble residue was washed thoroughly with cold water and was analyzed for phosphorus. When filter paper was present in the digestion, it was usually added as the pulp of one 9 cm. paper.

The results reported herein are the averages of closely agreeing duplicate determinations.

#### EFFECT OF FILTER PAPER

Egbert Janes (30), of the International Agricultural Corporation, advised the writers, in March 1935, that his laboratory had obtained markedly lower results for citrate-insoluble phosphorus in calcined phosphate when the sample was washed with water prior to the citrate digestion than when the washing was omitted and the sample was transferred directly from the weighing vessel to the citrate solution. He stated further that this discrepancy was not caused by the removal of water-soluble material during the preliminary washing, but it seemed to be due to the presence of filter paper during the digestion of the washed samples; filter paper was not present during the digestion of the unwashed samples. Also, Janes reported that asbestos fiber affected the solubility of calcined phosphate in a manner similar to that of filter paper, but fibrous materials had no effect on the solubility of ground phosphate rock. In this connection it should be pointed out that in order to facilitate the transfer of the sample to the hot citrate solution, it has always been the practice in the writers' laboratory to fold the sample (either washed or unwashed) into a filter paper. A search of the literature revealed no information concerning the effect of filter paper and asbestos on the solubility of phosphates.

The results given in Table 1 confirm Janes' observations, and they also show that the citrate solubility of calcined phosphate is increased by the presence of sand, but the effect is smaller than that of either paper or asbestos. In this connection it is interesting to note that Dubrisay (9) reported an increase in the citrate solubility of dicalcium phosphate in the presence of paraffin black; talc, kaolin, and paraffin black had a similar effect on the solubility of superphosphate, but kieselguhr had no effect. Dubrisay seems inclined to the opinion that the observed effects of these materials are due in some manner to selective absorption of ions during the citrate digestion.

Filter paper also has a marked effect in increasing the citrate solubility of basic slag, Rhenania phosphate, Non-acid phosphate, and certain of the possible components of calcined phosphate (Tables 2 and 3). On the other hand, filter paper has little or no effect on the solubility of

TABLE 1.—*Effect of filter paper, asbestos, and sand on solubility of calcined phosphate in neutral ammonium citrate solution*(Calcined phosphate No. 1374; total  $P_2O_5$  = 35.17%)

| MATERIAL ADDED <sup>a</sup>          | CITRATE-INSOLUBLE $P_2O_5$<br>per cent |
|--------------------------------------|--|
| None                                 | 7.01                                   |
| Sand, <sup>b</sup> 0.5 gram          | 6.68                                   |
| 1.0 gram                             | 5.95                                   |
| 2.0 grams                            | 5.80                                   |
| Asbestos fiber, 0.25 gram            | 5.30                                   |
| 0.50 gram                            | 4.11                                   |
| 1.00 gram                            | 3.88                                   |
| Filter paper, <sup>c</sup> 0.5 paper | 4.00                                   |
| 1.0 paper                            | 3.91                                   |
| 5.0 papers                           | 3.99                                   |

<sup>a</sup> The material was added to the hot citrate solution before the calcined phosphate was added.<sup>b</sup> —40 mesh, with comparatively little —80 mesh material.<sup>c</sup> 9 cm., Whatman No. 40.TABLE 2.—*Effect of filter paper on solubility of phosphates in neutral ammonium citrate solution*

| SAMPLE | MATERIAL  | TOTAL<br>$P_2O_5$<br>per cent | CITRATE-INSOLUBLE $P_2O_5$<br>per cent |                   | pH OF<br>CITRATE<br>EXTRACT <sup>a</sup> |
|--------|---|-------------------------------|--|-------------------|--|
|        |   |                               | WITHOUT<br>PAPER                       | WITH<br>PAPER     |  |
| 1374   | Calcined phosphate                              | 35.17                         | 7.01                                   | 3.91              | 7.8                                      |
| 1417   | Calcined phosphate                              | 33.30                         | 8.33                                   | 5.78              | 7.8                                      |
| 1351   | Calcined phosphate                              | 36.58                         | 13.67                                  | 11.80             | 7.8                                      |
| 1395   | Rhenania phosphate                              | 30.42                         | 8.85                                   | 3.70              | 8.2                                      |
| 1396   | Rhenania phosphate                              | 25.66                         | 6.70                                   | 2.18              | 8.4                                      |
| 1110   | Non-acid phosphate                              | 26.50                         | 13.33                                  | 11.42             | 7.9                                      |
| 1107   | Basic slag                                      | 23.36                         | 8.55                                   | 4.55              | 8.2                                      |
| 1164   | Basic slag                                      | 18.45                         | 6.10                                   | 3.74              | 8.1                                      |
| 1165   | Steamed bone meal <sup>b</sup>                  | 34.56                         | 20.32                                  | 19.76             | 7.8                                      |
| 1139   | Phosphate rock, South Carolina land             | 26.71                         | 22.04                                  | 21.72             | 7.2                                      |
| 790    | Phosphate rock, Florida land-pebble             | 31.39                         | 28.24                                  | 28.36             | 7.1                                      |
| 908    | Phosphate rock, Tennessee brown                 | 34.29                         | 31.95                                  | 32.36             | 7.2                                      |
| 1021   | Dicalcium phosphate                             | 40.20                         | 8.15                                   | 7.80              | 7.0                                      |
| 287    | Tricalcium phosphate, precipitated <sup>b</sup> | 40.86                         | 24.84                                  | 24.92             | 7.5                                      |
| 1197   | Tricalcium phosphate, precipitated <sup>b</sup> | 41.32                         | 12.10                                  | 11.91             | 7.8                                      |
| 1315   | Superphosphate <sup>c</sup>                     | 19.20                         | 0.11 <sup>d</sup>                      | 0.12 <sup>d</sup> | 6.5                                      |
| 1315   | Superphosphate                                  | 19.20                         | —                                      | 0.08 <sup>e</sup> | 6.8                                      |
| 1316   | Superphosphate <sup>f</sup>                     | 18.86                         | 0.12 <sup>d</sup>                      | 0.14 <sup>d</sup> | 6.5                                      |
| 1316   | Superphosphate                                  | 18.86                         | —                                      | 0.09 <sup>e</sup> | 6.9                                      |
| 1059   | Double superphosphate <sup>g</sup>              | 43.90                         | 0.79 <sup>d</sup>                      | 0.88 <sup>d</sup> | 6.3                                      |
| 1059   | Double superphosphate                           | 43.90                         | —                                      | 0.98 <sup>e</sup> | 6.7                                      |
| 1361   | Double superphosphate <sup>h</sup>              | 49.27                         | 0.19 <sup>d</sup>                      | 0.16 <sup>d</sup> | 6.3                                      |
| 1361   | Double superphosphate                           | 49.27                         | —                                      | 0.09 <sup>e</sup> | 6.7                                      |

<sup>a</sup> Filtered, undiluted extract.<sup>b</sup> Extract filtered through Chamberland-Pasteur tube.<sup>c</sup> Prepared from Florida land-pebble phosphate; contained 16.70% of water-soluble  $P_2O_5$ .<sup>d</sup> Determined directly on sample without removal of water-soluble  $P_2O_5$ .<sup>e</sup> Water-soluble  $P_2O_5$  removed before citrate digestion.<sup>f</sup> Prepared from Tennessee brown-rock phosphate; contained 12.30% of water-soluble  $P_2O_5$ .<sup>g</sup> Prepared from Tennessee brown-rock phosphate; contained 29.90% of water-soluble  $P_2O_5$ .<sup>h</sup> Prepared from Florida land-pebble phosphate; contained 41.63% of water-soluble  $P_2O_5$ .

TABLE 3.—*Effect of filter paper on solubility in neutral ammonium citrate solution of possible components of calcined phosphate*

| SAMPLE | MATERIAL   | TOTAL<br>P <sub>2</sub> O <sub>5</sub> | CITRATE-INSOLUBLE P <sub>2</sub> O <sub>5</sub> |                    | pH OF<br>CITRATE<br>EXTRACT <sup>a</sup> |
|--------|--|--|---|--------------------|--|
|        |  |  | WITHOUT<br>PAPER                                | WITH<br>PAPER      |  |
|        |  | <i>per cent</i>                        | <i>per cent</i>                                 | <i>per cent</i>    |  |
| 1400   | β-Tricalcium phosphate   | 45.52                                  | 23.47   | 21.50              | 7.4                                      |
| 1424   | α-Tricalcium phosphate   | 46.01                                  | 8.10  | 4.04               | 7.6                                      |
| 1397   | Hydroxyapatite   | 41.30                                  | —   | 31.66 <sup>b</sup> | —  |
| 1425   | Mixture of hydroxyapatite, 10 parts,<br>and quartz flour, 1 part, heated in<br>presence of water vapor at 1400°C. <sup>c</sup> | 39.33                                  | 3.78  | 1.15               | 7.7                                      |
| 1426   | Hydroxyapatite heated alone in dry air<br>at 1400°C. <sup>d</sup>  | 43.50                                  | 2.97  | 1.11               | 7.7                                      |
| 1478   | Calcined phosphate   | 37.25                                  | 5.75  | 3.73               | —  |
| 1478-H | Reverted calcined phosphate <sup>e</sup>   | 37.02                                  | 23.08   | 20.50              | —  |

<sup>a</sup> Filtered, undiluted extract.<sup>b</sup> Extract filtered through Chamberland-Pasteur tube; residue washed with 2% ammonium chloride solution.<sup>c</sup> The principal constituent possibly is α-tricalcium phosphate<sup>d</sup> The probable constituents are tetracalcium phosphate and principally α-tricalcium phosphate (760, 64).<sup>e</sup> Prepared by heating 50 gram samples of No. 1478 for 1 hour at 1000°C in the presence of water vapor.

steamed bone meal, ground phosphate rock, crude dicalcium phosphate, precipitated tricalcium phosphate, superphosphate, and double superphosphate (Table 2). These results indicate that the effect of filter paper on citrate solubility is confined principally to soluble phosphates produced by reactions at high temperatures; to a certain extent, the effect seems to increase with the solubility of the phosphate. On the other hand, the effect of filter paper on citrate solubility practically disappears when the flask is shaken continuously (Table 10).

The extent of the effect of the filter paper depends, within certain limits, upon the length of time that it is in contact with the phosphate during the citrate digestion (Table 4). Under the conditions of the official method, the maximum effect seems to be obtained with a contact time of about 50 minutes. Progressively higher figures for insoluble phosphorus are obtained with shorter periods of contact.

When samples of calcined phosphate, basic slag, bone meal, and ground phosphate rock are shaken continuously for 30 minutes with 2 per cent citric acid according to the official method for available phosphorus in basic slag, the results for citric acid-insoluble phosphorus are not affected by the presence of filter paper during the digestion (Table 5). On the

TABLE 4.—*Effect of time of contact with filter paper on solubility of calcined phosphate in neutral ammonium citrate solution*

| TIME OF DIGESTION BEFORE<br>PAPER <sup>a</sup> WAS ADDED | CITRATE-INSOLUBLE $P_2O_5$               |  |
|--|--|--|
|  | CALCINED PHOSPHATE NO. 1374 <sup>b</sup> | CALCINED PHOSPHATE NO. 1351 <sup>c</sup> |
| minutes  | per cent                                 | per cent                                 |
| 0  | 3.91                                     | 11.80                                    |
| 10   | 3.90                                     | 11.88                                    |
| 20   | 4.28                                     | 11.95                                    |
| 30   | 4.58                                     | 12.09                                    |
| 40   | 4.93                                     | 12.29                                    |
| 50   | 5.21                                     | 12.50                                    |
| 60 <sup>d</sup>  | 6.82                                     | 13.15                                    |
| No paper   | 7.01                                     | 13.67                                    |

<sup>a</sup> Paper added just before shaking the citrate solution. <sup>b</sup> Total  $P_2O_5$  = 35.17 %. <sup>c</sup> Total  $P_2O_5$  = 36.58 %.<sup>d</sup> Paper was in contact with the sample for a period of not more than 2 minutes before the citrate extract was completely filtered off.

other hand, filter paper has a marked effect on the results for citric acid-insoluble phosphorus in basic slag and calcined phosphate when the flasks are shaken by hand at intervals of 2.5 to 10 minutes (Table 9).

TABLE 5.—*Effect of filter paper on solubility of phosphates in 2 per cent citric acid solution*

| SAMPLE | MATERIAL                            | TOTAL<br>$P_2O_5$ | CITRIC ACID-INSOLUBLE $P_2O_5$ |               |
|--------|-------------------------------------|-------------------|--------------------------------|---------------|
|        |                                     |                   | WITHOUT<br>PAPER               | WITH<br>PAPER |
|        |                                     | per cent          | per cent                       | per cent      |
| 1374   | Calcined phosphate                  | 35.17             | 2.90                           | 2.80          |
| 1351   | Calcined phosphate                  | 36.58             | 10.92                          | 10.85         |
| 1396   | Rhenania phosphate                  | 25.66             | 1.48                           | 1.58          |
| 1164   | Basic slag                          | 18.45             | 2.87                           | 2.87          |
| 1165   | Steamed bone meal                   | 34.56             | 6.61                           | 6.52          |
| 790    | Phosphate rock, Florida land pebble | 31.39             | 24.48                          | 24.45         |
| 908    | Phosphate rock, Tennessee brown     | 34.29             | 28.39                          | 28.33         |

In general, the results do not seem to be affected significantly by variations in the quality of the filter paper present during the digestion. It is preferable, however, to use a paper that can be easily pulped in the solution. The evidence indicates that the action of filter paper, asbestos, etc., in promoting the solubility of certain phosphates under certain conditions is merely a mechanical effect. It is probable that the added material tends to prevent the agglomeration of the phosphate particles and to facilitate the contact between the solid and the solvent. There is no visible evidence of caking when either basic slag or calcined phosphate is digested in the absence of filter paper. Under the same conditions Rhe-

nania phosphate cakes considerably and tends to adhere rather tenaciously to the sides and bottom of the flask; the caking is reduced but not entirely eliminated in the presence of filter paper.

The official method for the determination of citrate-insoluble phosphorus classifies phosphate fertilizers into two broad groups, namely, "acidulated samples," which are washed with water prior to the citrate digestion, and "non-acidulated" samples (including the classification "precipitated phosphates"), which are digested without preliminary washing. The directions specify that "non-acidulated samples" shall be treated, without previous washing with water, as directed for "acidulated samples." The analyst may interpret the directions to mean either that the "non-acidulated sample" is transferred directly from the weighing vessel to the hot citrate solution and is digested in the absence of filter paper or that the sample is first transferred to a filter paper which, together with the sample, is added to the citrate solution. As the directions stand at present, the writers believe that either interpretation is permissible. In view of the results given in this paper it is evident, however, that the method should definitely specify either the omission of filter paper from or its inclusion in the citrate digestion.\*

#### EFFECT OF WASHING WITH WATER PRIOR TO CITRATE DIGESTION

The removal, prior to the citrate digestion, of the small amount of water-soluble material (0.30 to 6.50 per cent) seems to have little or no effect on the citrate solubility of the phosphorus in calcined phosphate, Rhenania phosphate, and basic slag (Table 6). Janes (30) found that prior

TABLE 6.—*Effect of washing with water prior to citrate digestion on solubility of phosphates in neutral ammonium citrate solution*  
(Filter paper present in all citrate digestions)

| SAMPLE | MATERIAL                           | TOTAL $P_2O_5$  | CITRATE-INSOLUBLE $P_2O_5$ |                 | TOTAL<br>$H_2O$ -SOLUBLE<br>MATERIAL |
|--------|------------------------------------|-----------------|----------------------------|-----------------|--------------------------------------|
|        |                                    |                 | WASHED                     | UNWASHED        |                                      |
|        |                                    | <i>per cent</i> | <i>per cent</i>            | <i>per cent</i> | <i>per cent</i>                      |
| 1416   | Calcined phosphate                 | 33.81           | 3.86                       | 3.89            | 0.33                                 |
| 1417   | Calcined phosphate                 | 33.30           | 5.70                       | 5.78            | 0.30                                 |
| 1419   | Calcined phosphate                 | 38.12           | 10.75                      | 10.59           | 0.33                                 |
| 1395   | Rhenania phosphate <sup>a</sup>    | 30.42           | 3.55                       | 3.70            | 1.90                                 |
| 1396   | Rhenania phosphate <sup>b</sup>    | 25.66           | 1.93                       | 2.18            | 2.34                                 |
| 1164   | Basic slag                         | 18.45           | 3.81                       | 3.74            | 6.50                                 |
| 1315   | Superphosphate <sup>c</sup>        | 19.20           | 0.08                       | 0.12            | —                                    |
| 1316   | Superphosphate <sup>d</sup>        | 18.86           | 0.09                       | 0.14            | —                                    |
| 1059   | Double superphosphate <sup>e</sup> | 43.90           | 0.98                       | 0.88            | —                                    |
| 1361   | Double superphosphate <sup>f</sup> | 49.27           | 0.09                       | 0.16            | —                                    |

<sup>a</sup> Water-soluble  $P_2O_5$  = 0.29 %.

<sup>b</sup> Water-soluble  $P_2O_5$  = 0.27 %.

<sup>c</sup> Water-soluble  $P_2O_5$  = 16.70 %.

<sup>d</sup> Water-soluble  $P_2O_5$  = 12.30 %.

<sup>e</sup> Water-soluble  $P_2O_5$  = 29.90 %.

<sup>f</sup> Water-soluble  $P_2O_5$  = 41.63 %.

\* See 1935 edition of *Methods of Analysis*, A.O.A.C., to issue September, 1936.

removal of the water-soluble material has no effect on the citrate solubility of calcined phosphate. It will be noted that nearly the same results for citrate-insoluble phosphorus in superphosphate and double superphosphate were obtained when the sample was digested directly as when the water-soluble phosphorus was removed prior to the citrate digestion. On the other hand, Austin (3) showed, more than a decade ago, that thorough washing with water, prior to the citrate digestion, is essential when 2 gram samples of either superphosphate or double superphosphate are digested with 100 cc. of citrate solution.

#### EFFECT OF pH OF CITRATE SOLUTION

The use of a neutral ammonium citrate solution for evaluating the phosphorus in water-insoluble phosphates was first proposed in 1871 by Fresenius, Neubauer, and Luck (10). Since that time, many investigators have shown that the citrate solubility of phosphates depends to a considerable extent on the reaction of the solution, the solubility of the calcium phosphates increasing with the acidity of the solution. This probably accounts for the discordant results reported by many of the early workers who had no accurate method for determining the true reaction of their solutions. Largely as a result of the excellent work of Robinson (45, 46), about 1919 to 1922, the reaction of citrate solutions can now be accurately determined and controlled by potentiometric and colorimetric methods. The effect of the pH of the citrate solution on the solubility of di- and tricalcium phosphates has been investigated by Robinson (45) and by Jacob, Rader, Marshall, and Beeson (26). Howes and Jacobs (22), and Andreasen and Raaschou (1) have studied the effect of pH on the citrate solubility of superphosphate and ammoniated superphosphate.

In 1886 Wagner (56) proposed the use of an acid ammonium citrate solution for the determination of available phosphorus in superphosphate and precipitated phosphate. For the evaluation of the phosphorus content of basic slag, he (57) recommended, in 1894, the use of a solution containing 60 grams of crystallized citric acid and 11.17 grams of ammonia per liter, the solution containing 14 grams of free citric acid per liter. In 1896 Gerlach and Passon (12) reported that in tests on 84 samples of basic slag the same results for soluble phosphorus were usually obtained by the use of a solution containing no ammonia and only 14 grams of citric acid per liter (corresponding to the free citric acid in Wagner's acid ammonium citrate solution) as by the use of acid ammonium citrate. They further reported that a solution containing 46 grams of citric acid and 11.17 grams of ammonia per liter (corresponding to Wagner's solution without the free citric acid) gave very much lower results than did either Wagner's acid ammonium citrate or a 1.4 per cent citric acid solution. They concluded that free citric acid and not ammonium citrate is the active solvent for phosphorus in basic slag. On the basis of the results of a large

number of plant-growth tests, Wagner (58) recommended, in 1899, the use of a 2 per cent citric acid solution for the determination of available phosphorus in basic slag. This modified method, which is described in detail in a later publication by Wagner, Dorsch, Aschoff, and Kunze (59), was adopted without change by the Association of Official Agricultural Chemists (18, 47) in 1922.

Since the publication of the paper by Gerlach and Passon (12) it seems to have been the general opinion that ammonium citrate solution is not a good solvent for the phosphorus in basic slag. Hackl (15) and Seidenstücker (52) state that basic slag is comparatively insoluble in neutral ammonium citrate solution. Wilhelmj, Karst, and Gericke (61) report that basic slag is much more soluble in 2 per cent citric acid than in ammonium citrate solution, whereas these reagents are about equally effective in dissolving the phosphorus of Rhenania phosphate. On the other hand, Jacob, Rader, and Ross (27) show that the solubility of basic slag in neutral ammonium citrate solution depends to a marked extent on the weight of sample used; when the digestion is made in the ratio of 0.5 gram of slag to 100 cc. of citrate solution the results for soluble phosphorus are usually close to those obtained by the 2 per cent citric acid method.

Graire (14) has investigated the solubility of basic slag, Rhenania phosphate and similar materials, bone ash, ammoniated superphosphate, ground phosphate rock, dicalcium phosphate, and superphosphate in acid, neutral, and alkaline ammonium citrate solutions. Unfortunately the concentration of citrate radical in the different solutions was not maintained at a constant value, and the results are not directly comparable because they represent the resultant effect of variations both in pH and in concentration of citrate radical.

In order to obtain information on the effect of pH on the solubility of basic slag, calcined phosphate, and other phosphates when the concentration of citrate radical is maintained constant, experiments were made with several solutions containing 188.13 grams of pure crystallized citric acid,  $C_6H_8O_7 \cdot H_2O$ , per liter<sup>1</sup> at 20°C. prepared as follows:

The citric acid (376.26 grams of the pure monohydrate) was dissolved in about 800 cc. of distilled water in a 2 liter volumetric flask, and the solution was roughly neutralized to the desired pH with strong ammonium hydroxide, care being taken not to overstep the desired pH. The reaction of the solution was then carefully adjusted colorimetrically to the desired value by the addition of small quantities of dilute ammonium hydroxide. All the test portions were returned to the flask, and the solution was diluted to 2 liters. The pH of the solution was finally determined potentiometrically by the use of the hydrogen electrode.<sup>2</sup>

<sup>1</sup> As an average of tests on 8 carefully prepared solutions, Robinson (45) found that 1 liter of a citrate solution having a pH of 7.0 and a specific gravity of 1.09 at 20°C. contains 188.13 grams of citric acid monohydrate, or 172 grams of the anhydrous acid.

<sup>2</sup> The determination was kindly made by E. F. Snyder of the Soil Fertility Division, Bureau of Plant Industry.



TABLE 7.—*Effect of pH of citrate solution on solubility of phosphates*  
(All citrate solutions contained the equivalent of 188.13 grams of citric acid monohydrate per liter)

| pH OF CITRATE SOLUTION                    | INSOLUBLE P <sub>2</sub> O <sub>5</sub> |                 | pH OF EXTRACT <sup>a</sup> |
|---|---|-----------------|----------------------------|
|   | WITHOUT PAPER                           | WITH PAPER      |                            |
| Calcined phosphate No. 1374 <sup>b</sup>  |   |                 |                            |
|   | <i>per cent</i>                         | <i>per cent</i> |                            |
| 7.5                                       | 6.94                                    | 3.94            | 8.0                        |
| 7.0                                       | 7.01                                    | 3.91            | 7.8                        |
| 6.6                                       | 5.44                                    | 3.97            | 7.6                        |
| 6.1                                       | 4.40                                    | 3.53            | 6.7                        |
| 5.0                                       | 3.13                                    | 2.65            | 5.0                        |
| Citric acid <sup>c</sup>                  | 2.90                                    | 2.80            | 3.4                        |
| Basic slag No. 1164 <sup>d</sup>          |   |                 |                            |
| 7.5                                       | 5.89                                    | 3.88            | 8.2                        |
| 7.0                                       | 6.10                                    | 3.74            | 8.1                        |
| 6.6                                       | 5.29                                    | 3.58            | 7.8                        |
| 6.1                                       | 4.02                                    | 2.95            | 7.5                        |
| 5.0                                       | 2.61                                    | 2.78            | 5.4                        |
| Citric acid <sup>c</sup>                  | 2.87                                    | 2.87            | 3.6                        |
| Rhenania phosphate No. 1396 <sup>e</sup>  |   |                 |                            |
| 7.5                                       | 7.23                                    | 1.65            | 8.5                        |
| 7.0                                       | 6.70                                    | 2.18            | 8.4                        |
| 6.6                                       | 6.08                                    | 2.19            | 7.8                        |
| 6.1                                       | 4.46                                    | 1.45            | 7.3                        |
| 5.0                                       | 2.84                                    | 1.60            | 5.0                        |
| Citric acid <sup>c</sup>                  | 1.48                                    | 1.58            | 3.9                        |
| Tricalcium phosphate No. 287 <sup>f</sup> |   |                 |                            |
| 7.5                                       | 25.71                                   | 25.29           | 7.5                        |
| 7.0                                       | 24.84                                   | 24.92           | 7.5                        |
| 6.6                                       | 21.12                                   | 21.37           | 7.0                        |
| 6.1                                       | 13.65                                   | 13.05           | 6.4                        |
| 5.0                                       | 0.49                                    | 0.22            | 5.0                        |
| Citric acid <sup>c</sup>                  | 3.12                                    | —               | 3.4                        |
| Phosphate rock No. 790 <sup>g</sup>       |   |                 |                            |
| 7.5                                       | 28.77                                   | 29.02           | —                          |
| 7.0                                       | 28.24                                   | 28.36           | 7.1                        |
| 6.6                                       | 27.64                                   | 27.57           | —                          |
| 6.1                                       | 26.56                                   | 26.16           | —                          |
| 5.0                                       | 22.70                                   | 21.13           | —                          |
| Citric acid <sup>c</sup>                  | 24.48                                   | 24.45           | 2.6                        |

<sup>a</sup> Filtered, undiluted extract.<sup>b</sup> Total  $P_2O_5$  = 35.17 %.<sup>c</sup> Two per cent solution, pH 2.05.<sup>d</sup> Total  $P_2O_5$  = 18.45 %.<sup>e</sup> Total  $P_2O_5$  = 25.66 %.<sup>f</sup> Precipitated material; total  $P_2O_5$  = 40.86 %.<sup>g</sup> Florida land pebble; total  $P_2O_5$  = 31.39 %.

The results (Table 7) indicate that when the citrate digestions are made in the presence of filter paper variations in  $pH$  within the range 6.6 to 7.5 have no effect on the citrate solubility of calcined phosphate and comparatively little effect on the solubility of basic slag; the solubility of these phosphates increases at lower  $pH$  values, and at  $pH$  5.0 it is slightly above the solubility in 2 per cent citric acid ( $pH$  2.05). In the presence of filter paper, the citrate solubility of Rhenania phosphate does not vary greatly in the  $pH$  range 5.0 to 7.5, and the solubility at  $pH$  7.5 is practically the same as the solubility in 2 per cent citric acid. On the other hand, the solubility of precipitated tricalcium phosphate and ground phosphate rock increases with decrease in  $pH$ ; these materials are much more soluble at about  $pH$  5.0 to 6.1 than at higher  $pH$  values, and they are more soluble in citrate solution at  $pH$  5.0 than in 2 per cent citric acid.

In the absence of filter paper, the citrate solubility of calcined phosphate, basic slag, and Rhenania phosphate increases markedly with decrease in  $pH$  value of the solution. Furthermore, the effect of filter paper in promoting the solubility of the phosphorus decreases with the  $pH$ , and at  $pH$  5.0 the results obtained in the presence of filter paper are fairly close to those obtained in its absence.

#### EFFECT OF PHOSPHATES ON THE $pH$ OF CITRATE AND CITRIC ACID SOLUTIONS

Robinson (45) reported that the digestion of di- and tricalcium phosphates and phosphate rock in citrate solutions having initial  $pH$  values of 6.6, 7.0, 7.4, and 7.8 caused a marked shift of  $pH$  in the acid and neutral solutions toward the alkaline side, whereas the  $pH$  values of the alkaline solutions were increased only slightly. On the other hand, commercial phosphates (presumably superphosphates) had little or no effect on the reaction of acid and neutral solutions but decreased the  $pH$  of alkaline solutions. As reported by Ross and Hardesty (48), Butt observed that digestion of calcined phosphate in citrate solutions having original  $pH$  values of 6.0 and 7.0 caused a marked increase in the  $pH$ , whereas only a small change was produced in the reaction of solutions having original  $pH$  values of 5.0 and 8.0. Bamberg (4) showed that digestion of 5 gram samples with 500 cc. of 2 per cent citric acid for 30 minutes increased the  $pH$  of the solution from 1.97 to 3.56–4.08 with basic slag, to 3.70–3.72 with Rhenania phosphate, and to only 2.64 with ground Gafsa phosphate rock.

As shown in Tables 2 and 3, digestion of phosphates in neutral ammonium citrate solution increased the  $pH$  value to 7.9–8.4 in the case of Non-acid phosphate, basic slag, and Rhenania phosphate and to 7.4–7.8 in the case of steamed bone meal, precipitated tricalcium phosphate, and calcined phosphate and certain of its possible components. Ground phos-

phate rock increased the *pH* to only 7.1–7.2 and dicalcium phosphate had no effect on the reaction of the solution. Superphosphate and double superphosphate decreased the *pH* to 6.3–6.9; as would be expected, the decrease was greater when the sample was digested without previous washing than when the water-soluble phosphorus was removed prior to the citrate digestion.

Calcined phosphate, basic slag, and Rhenania phosphate caused marked increases in the *pH* values of citrate solutions having original *pH* values of 6.1 to 7.5, but had little or no effect on a solution having an original *pH* of 5.0 (Table 7). These materials increased the *pH* of 2 per cent citric acid solution from 2.05 to 3.4–3.9.

The *pH* of the citrate extract was determined on the filtered, undiluted solution obtained by digesting the sample in the presence of filter paper. Except in the case of basic slags, the *pH* was determined colorimetrically and the results were corrected for the salt error. Because the extracts of basic slag were highly colored, the *pH* of these solutions was determined potentiometrically by means of the glass electrode;<sup>1</sup> the hydrogen electrode did not give satisfactory results on these solutions.

#### EFFECT OF WEIGHT OF SAMPLE

In 1881 König (31) pointed out that, with a constant volume of citrate solution, the solubility of precipitated phosphate varies with the weight of sample used. Since that time, the effect of sample weight on the solubility of phosphates in ammonium citrate and citric acid solutions has been studied by many investigators (6, 13, 14, 16, 17, 19, 22, 23, 25, 27, 36, 43, 44, 49, 53, 55).

As shown in Table 8, the figures for insoluble phosphorus in calcined

TABLE 8.—*Effect of weight of sample on solubility of calcined phosphate and basic slag in neutral ammonium citrate and 2 per cent citric acid solutions*  
(Filter paper present in citrate digestions, but absent in citric acid digestions)

| WEIGHT OF SAMPLE<br>PER 100 CC. OF<br>EXTRACTANT | CALCINED PHOSPHATE                                     |  |  |  | BASIC SLAG <sup>c</sup>                                |  |
|--|--|--|--|--|--|--|
|  | NO. 1351 <sup>a</sup>                                  |  | NO. 1374 <sup>b</sup>                                  |  |  |  |
|  | CITRATE-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> | CITRIC ACID-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> | CITRATE-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> | CITRIC ACID-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> | CITRATE-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> | CITRIC ACID-<br>INSOLUBLE<br>P <sub>2</sub> O <sub>5</sub> |
|  | gram   | per cent   | gram   | per cent   | gram   | per cent   |
| 1.00   | 11.80  | 10.92  | 3.91   | 2.90   | 3.74   | 2.87   |
| 0.75   | 11.65  | 10.97  | 3.52   | 2.86   | 3.45   | 2.70   |
| 0.50   | 11.57  | 10.83  | 3.47   | 2.99   | 3.20   | 2.25   |
| 0.25   | 11.50  | 10.07  | 3.39   | 2.58   | 2.88   | 1.55   |

<sup>a</sup> Total P<sub>2</sub>O<sub>5</sub> = 36.58 %.

<sup>b</sup> Total P<sub>2</sub>O<sub>5</sub> = 35.17 %.

<sup>c</sup> Total P<sub>2</sub>O<sub>5</sub> = 18.45 %.

<sup>1</sup> The determinations were kindly made by R. T. Milner of the Fertiliser Research Division, Bureau of Chemistry and Soils.

phosphate are not greatly affected by decreasing the sample weight from 1 gram to 0.25 gram per 100 cc. of extractant. Within these limits, the solubility of basic slag seems to be affected somewhat more by variations in the sample weight than is the solubility of calcined phosphate. Although the data are not sufficient to warrant definite conclusions, they indicate that all or nearly all the readily soluble phosphorus is dissolved from these materials by digesting 1 gram samples with 100 cc. of either neutral ammonium citrate or 2 per cent citric acid solution.

#### EFFECT OF SHAKING DURING DIGESTION OF THE SAMPLE

The official method for citrate-insoluble phosphorus specifies that the flask shall be shaken at the time the sample is added to the citrate solution and thereafter at the end of each 5 minute interval, or a total of 13 shaking periods. The method does not specify either how long or how vigorously the flask shall be shaken at each 5 minute interval. In the writers' laboratory it is the practice to shake the flasks vigorously, one at a time, for approximately 5 seconds with 8 to 10 oscillations. This is probably more vigorous agitation than is customary in most laboratories, but even then the total time of shaking is only about 65 seconds during the 1 hour digestion period. The effect of variations in the interval of shaking does not seem to have been previously studied.

When the digestions are made in the presence of filter paper, variations of 1 to 10 minutes in the interval of shaking seem to have an insignificant effect on the results for citrate-insoluble phosphorus in calcined phosphate whereas the solubility of basic slag seems to decrease somewhat when the interval of shaking is increased beyond about 2.5 minutes (Table 9). The citric-acid solubility of these materials tends to decrease with increase in the interval of shaking. Except when the flask was shaken continuously, the citric acid digestions were made in Erlenmeyer flasks of sufficient size to permit vigorous agitation of the solution.

In the absence of filter paper, the citrate and citric acid solubilities of both calcined phosphate and basic slag are markedly affected by variations in the interval of shaking, and for a given interval of shaking the results for insoluble phosphorus are much higher than those obtained in the presence of filter paper (Table 9). However, when the flask is shaken continuously the results are nearly the same whether or not filter paper is present during the digestion (Tables 9 and 10). Furthermore, shaking at 5 minute intervals with paper gives, with a few exceptions, about the same results as does continuous shaking (Table 10).

The official method for the determination of available phosphorus by the 2 per cent citric acid method specifies that the flask shall be rotated at the rate of 30 to 40 r.p.m. Several investigators (6, 11, 12, 59, 60) have studied the effect of speed of rotation of the flask on the solubility of basic slag. Wagner and co-workers (59, 60) reported that the results

TABLE 9.—*Effect of interval of shaking on solubility of calcined phosphate and basic slag in neutral ammonium citrate and 2 per cent citric acid solutions*

| INTERVAL OF SHAKING  | TOTAL TIME<br>OF SHAKING | CALCINED PHOSPHATE NO. 1374 <sup>a</sup> |               | BASIC SLAG NO. 1164 <sup>b</sup> |               |
|--|--------------------------|--|---------------|----------------------------------|---------------|
|  |                          | WITHOUT<br>PAPER                         | WITH<br>PAPER | WITHOUT<br>PAPER                 | WITH<br>PAPER |
| minutes  | minutes                  | per cent                                 | per cent      | per cent                         | per cent      |
| Citrate-insoluble P <sub>2</sub> O <sub>5</sub> <sup>c</sup>     |                          |  |               |                                  |               |
| Continuous shaking <sup>d</sup>                                  | 60.00                    | 3.74                                     | 3.79          | 3.48                             | 3.47          |
| 1.0  | 5.08                     | 4.05                                     | 3.80          | 3.75                             | 3.38          |
| 2.5  | 2.08                     | 4.53                                     | 3.88          | 5.30                             | 3.50          |
| 5.0  | 1.08                     | 7.01                                     | 3.91          | 6.10                             | 3.74          |
| 10.0   | 0.51                     | 9.28                                     | 3.90          | 6.76                             | 4.02          |
| 60.0 <sup>e</sup>  | 0.17                     | —  | 6.35          | —                                | 5.63          |
| Citric acid-insoluble P <sub>2</sub> O <sub>5</sub> <sup>f</sup> |                          |  |               |                                  |               |
| Continuous shaking <sup>g</sup>                                  | 30.00                    | 2.90                                     | 2.80          | 2.87                             | 2.87          |
| 2.5  | 1.08                     | 5.22                                     | 2.74          | 5.04                             | 3.09          |
| 5.0  | 0.51                     | 7.55                                     | 3.11          | 5.87                             | 3.16          |
| 10.0   | 0.33                     | 9.51                                     | 3.24          | 6.68                             | 5.73          |

<sup>a</sup> Total P<sub>2</sub>O<sub>5</sub> = 35.17 %.<sup>b</sup> Total P<sub>2</sub>O<sub>5</sub> = 18.45 %.<sup>c</sup> Time of digestion = 60 minutes.<sup>d</sup> End-over-end rotation (12 r.p.m.) in thermostat.<sup>e</sup> Shaken only at the time the sample was added to the citrate solution and at the end of the digestion.<sup>f</sup> Time of digestion = 30 minutes.<sup>g</sup> End-over-end rotation (18 r.p.m.).TABLE 10.—*Effect of continuous shaking on solubility of phosphates in neutral ammonium citrate solution*

| SAMPLE | MATERIAL           | TOTAL<br>P <sub>2</sub> O <sub>5</sub> | CITRATE-INSOLUBLE P <sub>2</sub> O <sub>5</sub> |               |                                 |               |
|--------|--------------------|--|---|---------------|---------------------------------|---------------|
|        |                    |  | SHAKING AT 5 MINUTE<br>INTERVALS                |               | CONTINUOUS SHAKING <sup>a</sup> |               |
|        |                    |  | WITHOUT<br>PAPER                                | WITH<br>PAPER | WITHOUT<br>PAPER                | WITH<br>PAPER |
|        |                    | per cent                               | per cent  | per cent      | per cent                        | per cent      |
| 1374   | Calcined phosphate | 35.17                                  | 7.01  | 3.91          | 3.74                            | 3.79          |
| 1351   | Calcined phosphate | 36.58                                  | 13.67   | 11.80         | 11.60                           | 11.79         |
| 1395   | Rhenania phosphate | 30.42                                  | 8.85  | 3.70          | 0.70                            | 0.43          |
| 1396   | Rhenania phosphate | 25.66                                  | 6.70  | 2.18          | 2.11                            | 1.84          |
| 1110   | Non-acid phosphate | 26.50                                  | 13.33   | 11.42         | 11.11                           | 11.00         |
| 1164   | Basic slag         | 18.45                                  | 6.10  | 3.74          | 3.48                            | 3.47          |
| 1107   | Basic slag         | 23.36                                  | 8.55  | 4.55          | 3.35                            | 3.38          |

<sup>a</sup> End-over-end rotation (12 r.p.m.) in thermostat.

are not affected by variations in the speed of rotation within the limits 25 to 45 r.p.m. Experiments do not seem to have been made at lower speeds. However, the data in Table 9 indicate that with continuous shaking the speed of rotation is not an important factor when the digestion is made in the presence of filter paper.

#### EFFECT OF PARTICLE SIZE

The effect of the fineness of the sample on the solubility of basic slag, phosphate rock, and precipitated di- and tricalcium phosphates has been studied by several investigators (6, 14, 16, 20, 21, 26, 38, 42). However, the majority of the experiments were made on samples that varied considerably in their mechanical composition and represented wide ranges of particle size. Hoffmeister (21) reported that when samples of ground basic slag were separated into different particle sizes the chemical composition of the fractions and the solubility of the phosphorus in Wagner's acid ammonium citrate solution varied with the particle size, the solubility increasing with decrease in particle size. Popp (38) also showed that the chemical composition of the mechanical fractions of ground basic slag varies with the particle size; he reported, however, that the solubility of the phosphorus in 2 per cent citric acid did not vary greatly with the different fractions.

The data given in Table 12 indicate that grinding the composite sample finer than about 80- to 100-mesh has comparatively little effect on the citrate solubility of the phosphorus of calcined phosphate. It will be noted that the 80-mesh samples contained considerable quantities of 200-mesh material (Table 11). Also, the results (Table 12) indicate that the

TABLE 11.—*Mechanical composition of calcined phosphate ground to different degrees of fineness*

| FINENESS OF GRINDING        |        | MECHANICAL COMPOSITION, MESH |          |          |           |            |            |          |
|-----------------------------|--------|------------------------------|----------|----------|-----------|------------|------------|----------|
|                             |        | 20 to 40                     | 40 to 60 | 60 to 80 | 80 to 100 | 100 to 150 | 150 to 200 | —200     |
| mesh                        | mm.    | per cent                     | per cent | per cent | per cent  | per cent   | per cent   | per cent |
| Calcined phosphate No. 1374 |        |                              |          |          |           |            |            |          |
| — 40                        | —0.381 | —                            | 11.4     | 8.6      | 8.9       | 14.3       | 14.8       | 42.0     |
| — 60                        | —0.221 | —                            | —        | 7.4      | 12.6      | 16.9       | 17.1       | 46.0     |
| — 80                        | —0.175 | —                            | —        | —        | 8.3       | 14.2       | 19.2       | 58.3     |
| —100                        | —0.147 | —                            | —        | —        | —         | 9.6        | 18.3       | 72.1     |
| —150                        | —0.104 | —                            | —        | —        | —         | —          | 29.5       | 70.5     |
| Calcined phosphate No. 1478 |        |                              |          |          |           |            |            |          |
| — 20                        | —0.833 | 50.2                         | 24.8     | 7.7      | 4.3       | 4.5        | 3.3        | 5.2      |
| — 40                        | —0.381 | —                            | 25.7     | 17.3     | 10.5      | 11.3       | 9.7        | 25.5     |
| — 80                        | —0.175 | —                            | —        | —        | 9.7       | 22.5       | 20.0       | 47.8     |

TABLE 12.—*Effect of fineness of grinding on solubility of calcined phosphate in neutral ammonium citrate and 2 per cent citric acid solutions*

| FINENESS OF GRINDING                     | CITRATE-INSOLUBLE $P_2O_5^a$             |  | CITRIC ACID<br>INSOLUBLE $P_2O_5^c$ |
|--|--|--|-------------------------------------|
|  | SOLUTION SHAKEN AT<br>5 MINUTE INTERVALS | SOLUTION SHAKEN<br>CONTINUOUSLY <sup>b</sup> |                                     |
| mesh                                     | per cent                                 | per cent                                     | per cent                            |
| Calcined phosphate No. 1374 <sup>d</sup> |  |  |                                     |
| — 40                                     | 4.80                                     | 4.58   | 3.67                                |
| — 60                                     | 4.10                                     | 4.05   | 2.99                                |
| — 80                                     | 3.91                                     | 3.79   | 2.90                                |
| — 100                                    | 3.50                                     | 3.56   | 2.86                                |
| — 150                                    | 3.55                                     | 3.47   | 2.74                                |
| — 200                                    | 3.44                                     | 3.48   | 2.90                                |
| Calcined phosphate No. 1478 <sup>e</sup> |  |  |                                     |
| — 20                                     | 7.80                                     | —  | 4.74                                |
| — 40                                     | 4.72                                     | —  | 3.06                                |
| — 80                                     | 3.73                                     | —  | 2.77                                |
| — 200                                    | 3.68                                     | —  | 2.78                                |

<sup>a</sup> Filter paper present in citrate digestions.<sup>b</sup> End-over-end rotation (12 r.p.m.) in thermostat.<sup>c</sup> Without filter paper.<sup>d</sup> Total  $P_2O_5$  = 35.17 %.<sup>e</sup> Total  $P_2O_5$  = 37.25 %.

solubility of calcined phosphate in 2 per cent citric acid is affected to only a small extent by grinding the sample finer than about 40- to 60-mesh.

In order to obtain information on the effect of particle size on the solubility of phosphates, samples of calcined phosphate and Non-acid phosphate were ground to pass a 20-mesh sieve and were carefully separated into several ranges of particle size. It is obvious that the specific effect of particle size on solubility can be determined only when all the particles are identical in physical structures and chemical composition. Complete analyses of the fractions were not made, but it will be noted that for a given material, particularly the calcined phosphates, the fractions do not show large variations in the total phosphorus content (Table 13). The variations in the percentages of citrate- and citric acid-insoluble phosphorus in the fractions of the calcined phosphates, after they were ground to pass a 200-mesh sieve, seem to be roughly correlated with the percentages of fluorine in the fractions.<sup>1</sup> It has been shown that the solubility of calcined phosphate in neutral ammonium citrate solution depends, more or less directly, on the extent to which the second half of the fluorine is volatilized from the phosphate rock (34, 39, 40). In the case of the Non-acid phosphate the total phosphorus and the citrate-

<sup>1</sup> The fluorine determinations were kindly made by D. S. Reynolds.

TABLE 13.—*Effect of particle size on solubility of phosphates in neutral ammonium citrate and 2 per cent citric acid solutions*

| mesh                        | PARTICLE SIZE  | TOTAL P <sub>2</sub> O <sub>5</sub> | TOTAL FLUORINE | CITRATE-SOLUBLE P <sub>2</sub> O <sub>5</sub> |                    |  |                              | CITRIC ACID-INSOLUBLE P <sub>2</sub> O <sub>5</sub> <sup>b</sup> |          |
|-----------------------------|----------------|-------------------------------------|----------------|---|--------------------|--|------------------------------|--|----------|
|                             |                |                                     |                | ORIGINAL MECHANICAL FRACTION                  |                    | SAMPLE GROUND TO 200 MESH <sup>a</sup> | ORIGINAL MECHANICAL FRACTION | SAMPLE GROUND TO 200 MESH <sup>a</sup>                           |          |
|                             |                |                                     |                | WITHOUT PAPER                                 |                    |  |                              |  |          |
|                             |                |                                     |                | per cent                                      | per cent           |  |                              |  | per cent |
| Calced phosphate No. 1374   |                |                                     |                |   |                    |  |                              |  |          |
| Composite sample            |                |                                     |                |   |                    |  |                              |  |          |
| 20 to 40                    | 0.833 to 0.381 | 35.17                               | 0.15           | 7.01 <sup>c</sup>                             | 3.91 <sup>c</sup>  | 3.44                                   | 2.90 <sup>c</sup>            | 2.90   |          |
| 40 to 60                    | 0.381 to 0.221 | 34.77                               | 0.11           | 17.10   | 12.20              | 3.58                                   | 7.09                         | 2.57   |          |
| 60 to 80                    | 0.221 to 0.175 | 34.72                               | 0.12           | 17.73   | 8.83               | 3.65                                   | 5.12                         | 2.88   |          |
| 80 to 100                   | 0.175 to 0.147 | 34.83                               | 0.14           | 14.63   | 6.84               | 3.94                                   | 3.97                         | 3.08   |          |
| 100 to 150                  | 0.147 to 0.104 | 34.82                               | 0.15           | 13.28   | 5.43               | 4.11                                   | 3.68                         | 3.03   |          |
| 150 to 200                  | 0.104 to 0.074 | 34.94                               | 0.14           | 10.35   | 4.64               | 4.19                                   | 3.37                         | 3.10   |          |
|                             |                | 35.21                               | 0.14           | 6.75  | 3.66               | 3.77                                   | 3.04                         | 2.89   |          |
| -200                        | -0.074         | 35.94                               | 0.12           | 3.78  | 3.12               | 3.12                                   | 2.35                         | 2.35   |          |
| Calced phosphate No. 1478   |                |                                     |                |   |                    |  |                              |  |          |
| Composite sample            |                |                                     |                |   |                    |  |                              |  |          |
| 20 to 40                    | 0.833 to 0.381 | 37.25                               | 0.09           | 5.75 <sup>c</sup>                             | 3.73 <sup>c</sup>  | 3.68                                   | 2.77 <sup>c</sup>            | 2.78   |          |
| 40 to 60                    | 0.381 to 0.221 | 37.30                               | 0.05           | 18.35   | 9.53               | 2.64                                   | 5.29                         | 1.82   |          |
| 60 to 80                    | 0.221 to 0.175 | 37.20                               | 0.15           | 12.70   | 5.63               | 4.92                                   | 4.58                         | 4.36   |          |
| 100 to 150                  | 0.147 to 0.104 | 37.12                               | 0.13           | 7.73  | 4.46               | 4.29                                   | 3.44                         | 3.56   |          |
| Non-acid phosphate No. 1110 |                |                                     |                |   |                    |  |                              |  |          |
| Composite sample            |                |                                     |                |   |                    |  |                              |  |          |
| 20 to 40                    | 0.833 to 0.381 | 26.50                               | 2.13           | 13.33 <sup>c</sup>                            | 11.42 <sup>c</sup> | 10.43                                  | —                            | —  |          |
| 40 to 60                    | 0.381 to 0.221 | 27.14                               | 2.12           | 21.82   | 19.85              | 11.28                                  | —                            | —  |          |
| 60 to 80                    | 0.221 to 0.175 | 26.74                               | 2.15           | 19.46   | 15.48              | 10.87                                  | —                            | —  |          |
| 80 to 100                   | 0.175 to 0.147 | 26.54                               | 2.21           | 17.70   | 13.42              | 10.89                                  | —                            | —  |          |
| 100 to 150                  | 0.147 to 0.104 | 26.27                               | 2.09           | 16.48   | 12.83              | 10.74                                  | —                            | —  |          |
| 150 to 200                  | 0.104 to 0.074 | 25.80                               | 2.03           | 14.02   | 11.60              | 10.54                                  | —                            | —  |          |
|                             |                | 25.13                               | 2.08           | 11.32   | 10.35              | 9.98                                   | —                            | —  |          |
| -200                        | -0.074         | 25.23                               | 2.14           | 9.58  | 9.61               | 9.61                                   | —                            | —  |          |

\* Filter paper present in citrate digestion.

b Without filter paper.

c Sample ground to 80-mesh.



insoluble phosphorus in the fractions after they were ground to 200-mesh tended, in general, to decrease with decrease in particle size. There is no evidence of a correlation between the solubility of the phosphorus and the fluorine content of the different fractions of this material, and a high degree of fluorine removal does not seem to be necessary for the formation of citrate-soluble phosphorus.

The results indicate, in general, that the variations in the chemical composition of the particles of a particular sample are not sufficient to cause marked changes in the solubility of the different fractions. If the variations in the physical structure of the particles, which are likely slight, are disregarded, it appears that the citrate solubility of calcined phosphate and Non-acid phosphate is greatly affected by the particle size of the material (Table 13). Although the effect is greatest in the coarser particles, as would be expected, the percentage of citrate-insoluble phosphorus decreases with the particle size throughout the ranges studied. The results indicate, however, that the effects of particle size are comparatively small in the fractions finer than about 100-mesh. In general, the effects of particle size in the citrate digestion are much more pronounced when paper pulp is absent than when it is present.

Particle size has decidedly less effect on the citric acid solubility than on the ammonium citrate solubility of calcined phosphate (Table 13). Although the percentages of citric acid-insoluble phosphorus decrease with particle size throughout the ranges studied, the effects of particle size are comparatively small in the fractions finer than about 60-mesh.

The official directions for the preparation of fertilizer samples for analysis (37) specify that the sample shall be ground to pass a sieve having circular openings 1 mm. in diameter. The openings in such a sieve are larger than those (0.833 mm.) in a 20-mesh sieve, which was the coarsest sieve used in obtaining the data reported in Tables 12 and 13. A sample of calcined phosphate ground to pass a sieve having openings 1 mm. in diameter will show a much higher content of insoluble phosphorus than will the same sample when ground to pass a sieve with smaller openings. The question then arises as to whether calcined phosphate should be coarsely ground or finely ground for the determination of available phosphorus. This question can be settled only on the basis of the results of plant-growth tests with different particle sizes of material. Such tests are now under way, and the results will be reported at the 1936 meeting of the Association of Official Agricultural Chemists. If it is shown that plants can utilize the phosphorus of coarsely ground calcined phosphate as effectively as that of the finely ground material, it would seem logical to use a sieve having openings smaller than 1 mm. in diameter in preparing the sample for analysis.

## EFFECT OF TEMPERATURE OF CITRATE SOLUTION

Huston and Jones (23) reported that the solubility of basic slag in neutral ammonium citrate solution was not greatly affected by varying the temperature of digestion within the limits of 50° and 85°C.; however, the time of digestion was 5 hours. As shown in Table 14, the citrate solubility of calcined phosphate and basic slag increases with the temperature of the digestion in the range 20° to 85°C. However, temperatures in excess of 65°C. seem to have a comparatively small effect on the results. The effect of temperature is not so great when the flask is shaken continuously as when it is shaken at 5 minute intervals. According to Wagner and co-workers (59), a variation of 1° C. in the temperature at which basic slag is digested in 2 per cent citric acid solution causes an average variation of 0.04 per cent in the results for soluble phosphoric oxide.

TABLE 14.—*Effect of temperature on solubility of calcined phosphate and basic slag in neutral ammonium citrate solution*

(Filter paper present in all citrate digestions)

| TEMPERATURE | CITRATE-INSOLUBLE $P_2O_5$               |                                     |                                  |                                     |
|-------------|--|-------------------------------------|----------------------------------|-------------------------------------|
|             | CALCINED PHOSPHATE NO. 1374 <sup>a</sup> |                                     | BASIC SLAG NO. 1164 <sup>b</sup> |                                     |
|             | SHAKEN AT 5<br>MINUTE INTERVALS          | SHAKEN<br>CONTINUOUSLY <sup>c</sup> | SHAKEN AT 5<br>MINUTE INTERVALS  | SHAKEN<br>CONTINUOUSLY <sup>c</sup> |
| °C.         | per cent                                 | per cent                            | per cent                         | per cent                            |
| 20          | 10.20                                    | 8.80                                | 7.63                             | 7.23                                |
| 45          | 5.80                                     | 4.44                                | 5.08                             | 4.08                                |
| 65          | 3.91                                     | 3.79                                | 3.74                             | 3.47                                |
| 85          | 3.58                                     | —                                   | 3.30                             | —                                   |

<sup>a</sup> Total  $P_2O_5$  = 35.17%. <sup>b</sup> Total  $P_2O_5$  = 18.45%. <sup>c</sup> End-over-end rotation (12 r.p.m.) in thermostat

## EFFECT OF TIME OF DIGESTION IN CITRATE SOLUTION

The effect of the time of digestion on the solubility of phosphates in neutral ammonium citrate solution has been studied by several investigators (14, 22, 23, 26, 49). In general, the results show that the figures for insoluble phosphorus in tricalcium phosphate, basic slag, superphosphate, ammoniated superphosphate, and ground phosphate rock decrease somewhat when the time of digestion is increased from 0.5 hour to 1 hour. Increasing the time of digestion beyond 0.5 hour seems to have comparatively little effect on the solubility of basic slag in 2 per cent citric acid solution (6, 11, 12, 59).

As shown in Table 15, the citrate-insoluble phosphorus in calcined phosphate and basic slag decreases with increase in the time of digestion, within the limits, 30 to 75 minutes. When the flask is shaken at 5 minute intervals the greatest decrease in insoluble phosphorus, per 15 minute

increase in the time of digestion, occurs on the interval between 30 and 45 minutes; the effect of time is much smaller when filter paper is present during the citrate digestion than when it is absent, and in the presence of filter paper increasing the time of digestion beyond 45 minutes has comparatively little effect on the figures for insoluble phosphorus. When the flask is shaken continuously, the presence of filter paper during the citrate digestion also has comparatively little effect on the results.

TABLE 15.—*Effect of time of digestion on solubility of calcined phosphate and basic slag in neutral ammonium citrate solution*

(Samples digested at 65°C.)

| TIME                                     | CITRATE-INSOLUBLE $P_2O_5$   |            |                                  |            |
|--|------------------------------|------------|----------------------------------|------------|
|  | SHAKEN AT 5 MINUTE INTERVALS |            | SHAKEN CONTINUOUSLY <sup>a</sup> |            |
|  | WITHOUT PAPER                | WITH PAPER | WITHOUT PAPER                    | WITH PAPER |
| minutes                                  | per cent                     | per cent   | per cent                         | per cent   |
| Calcined phosphate No. 1374 <sup>b</sup> |                              |            |                                  |            |
| 30                                       | 10.03                        | 4.68       | 4.70                             | 4.43       |
| 45                                       | 7.59                         | 4.19       | 4.08                             | 4.04       |
| 60                                       | 7.01                         | 3.91       | 3.74                             | 3.79       |
| 75                                       | 5.30                         | 3.80       | 3.44                             | 3.36       |
| Basic slag No. 1164 <sup>c</sup>         |                              |            |                                  |            |
| 30                                       | 7.49                         | 4.65       | 4.40                             | 4.09       |
| 45                                       | 6.40                         | 3.87       | 3.83                             | 3.75       |
| 60                                       | 6.10                         | 3.74       | 3.48                             | 3.47       |
| 75                                       | 5.03                         | 3.54       | 3.04                             | 3.05       |

<sup>a</sup> End-over-end rotation (12 r.p.m.) in thermostat. <sup>b</sup> Total  $P_2O_5$  = 35.17 %. <sup>c</sup> Total  $P_2O_5$  = 18.45 %.

#### COMPARISON OF NEUTRAL AMMONIUM CITRATE AND 2 PER CENT CITRIC ACID AS SOLVENTS FOR PHOSPHATES

Comparative results on 9 samples of calcined phosphate show 0.9 to 3.4 per cent more of insoluble phosphoric oxide by the neutral ammonium citrate method than by the 2 per cent citric acid method, the average difference being 1.8 per cent of the sample (Table 16). With 2 samples each of Rhenania phosphate and basic slag the results for insoluble phosphoric oxide average 2.1 and 1.8 per cent higher, respectively, by the citrate method than by the citric acid method. Reverted calcined phosphate, steamed bone meal, hydroxyapatite,  $\beta$ -tricalcium phosphate, and precipitated tricalcium phosphate are much more soluble in citric acid than in ammonium citrate solution.

TABLE 16.—*Comparison of neutral ammonium citrate and 2 per cent citric acid solutions as solvents for phosphates*

(Filter paper present in citrate digestions, but absent in citric acid digestions)

| SAMPLE | MATERIAL                                 | P <sub>2</sub> O <sub>5</sub> |                       |                       |
|--------|--|-------------------------------|-----------------------|-----------------------|
|        |  | TOTAL                         | CITRATE-INSOLUBLE     | CITRIC ACID-INSOLUBLE |
|        |  | per cent                      | per cent              | per cent              |
| 1416   | Calcined phosphate                       | 33.81                         | 3.89                  | 2.57                  |
| 1374   | Calcined phosphate                       | 35.17                         | 3.91                  | 2.90                  |
| 1420   | Calcined phosphate                       | 34.20                         | 5.18                  | 3.59                  |
| 1421   | Calcined phosphate                       | 35.31                         | 5.40                  | 3.37                  |
| 1417   | Calcined phosphate                       | 33.30                         | 5.78                  | 2.68                  |
| 1418   | Calcined phosphate                       | 34.05                         | 6.08                  | 2.66                  |
| 1419   | Calcined phosphate                       | 38.12                         | 10.59                 | 8.57                  |
| 1351   | Calcined phosphate                       | 36.58                         | 11.80                 | 10.92                 |
| 1478   | Calcined phosphate                       | 37.25                         | 3.73                  | 2.77                  |
| 1478-H | Reverted calcined phosphate <sup>a</sup> | 37.02                         | 20.50                 | 13.87                 |
| 1397   | Hydroxyapatite                           | 41.30                         | 31.66 <sup>b, c</sup> | 8.00                  |
| 1400   | $\beta$ -Tricalcium phosphate            | 45.52                         | 21.50                 | 11.67                 |
| 1424   | $\alpha$ -Tricalcium phosphate           | 46.01                         | 4.04                  | 2.08                  |
| 287    | Tricalcium phosphate, precipitated       | 40.86                         | 24.92 <sup>b</sup>    | 3.12                  |
| 1197   | Tricalcium phosphate, precipitated       | 41.32                         | 11.91 <sup>b</sup>    | 0.51                  |
| 1395   | Rhenania phosphate                       | 30.42                         | 3.70                  | 0.18                  |
| 1396   | Rhenania phosphate                       | 25.66                         | 2.18                  | 1.48                  |
| 1107   | Basic slag                               | 23.36                         | 4.55                  | 1.79                  |
| 1164   | Basic slag                               | 18.45                         | 3.74                  | 2.87                  |
| 1165   | Steamed bone meal                        | 34.56                         | 19.76 <sup>b</sup>    | 6.61                  |
| 790    | Phosphate rock, Florida land-pebble      | 31.39                         | 28.24                 | 24.48                 |
| 908    | Phosphate rock, Tennessee brown          | 34.29                         | 31.95                 | 28.39                 |

<sup>a</sup> Prepared by heating 50 gram samples of No. 1478 for one hour at 1000°C. in the presence of water vapor.<sup>b</sup> Citrate extract filtered through Chamberland-Pasteur tube<sup>c</sup> Citrate-insoluble residue washed with 2% ammonium chloride solution.

## SUMMARY

A study was made of the solubilities of calcined phosphate (prepared by heating phosphate rock at high temperatures in the presence of water vapor), basic slag, and other water-insoluble phosphates in ammonium citrate and citric acid solutions, as affected by the presence of filter paper during the digestion of the sample, the pH of the citrate solution, the fineness of the sample, the time and temperature of the digestion, the weight of the sample, and the amount of shaking during the digestion.

When the determinations are made according to the official method, the results for citrate-insoluble phosphorus in calcined phosphate and certain of its possible components, basic slag, and Rhenania phosphate and similar products obtained by heating phosphate rock with alkali salts, are significantly lower when filter paper is present during the citrate digestion than when it is absent; asbestos fiber has a similar effect. This effect,

however, disappears when the flask is shaken continuously during the citrate digestion. Also, filter paper has little or no effect on the citrate solubility of either precipitated tricalcium phosphate, commercial dicalcium phosphate, steamed bone meal, superphosphate, double superphosphate or ground phosphate rock, whether the flask is shaken continuously or at 5 minute intervals.

When the flask is shaken continuously for 30 minutes, filter paper has little or no effect on the solubility of phosphates in 2 per cent citric acid solution. When the flask is shaken at 2.5 to 10 minute intervals the results for citric acid-insoluble phosphorus are much lower in the presence of filter paper than in its absence.

The action of filter paper, asbestos, etc., in increasing the solubility of phosphates under certain conditions seems to be merely a mechanical effect. The fibrous material probably tends to prevent the agglomeration of the phosphate particles and to facilitate the contact between the solid and the solvent. The official method for the determination of citrate-insoluble phosphorus in "non-acidulated" samples should definitely specify either the omission or the inclusion of filter paper in the citrate digestion.

When the digestions are made in the presence of filter paper and the concentration of the citrate radical is maintained at a constant value (188.13 grams of citric acid monohydrate per liter), variations in the acidity of the solution within the limits pH 6.1 and 7.5 have comparatively little effect on the solubilities of calcined phosphate, basic slag, and Rhenania phosphate in ammonium citrate solutions. The solubilities of these materials in citrate solution of pH 5.0 are close to their solubilities in 2 per cent citric acid solution.

The solubility of calcined phosphate in neutral ammonium citrate solution is markedly affected by the particle size of the material, at least within the range of 20- to 200-mesh (0.833 to 0.074 mm.); the effect is greatest in the coarser particles. Grinding the sample finer than about 80- to 100-mesh has comparatively little effect on the citrate solubility of the phosphorus when the analyses are made on the composite samples. Particle size has far less effect on the citric acid solubility than on the ammonium citrate solubility of calcined phosphate.

In general, variations in the analytical procedure have less effect on the solubility of phosphates in 2 per cent citric acid than in neutral ammonium citrate solution. The behavior of calcined phosphate in both solvents is similar to that of basic slag.

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## THE PHOTOELECTRIC TURBIDIMETER AND THE ALKALINE TITRATION METHOD FOR THE DETERMINATION OF SMALL AMOUNTS OF HYDROCYANIC ACID\*

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### INTRODUCTION

The determination of the hydrocyanic acid content of biological materials has been the object of numerous investigations. Work has been done with a two-fold purpose, namely, to find suitable methods of analysis and to determine the actual cyanide content of the tissues.

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\* Paper No. 343, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

Numerous methods for the determination of small amounts of hydrocyanic acid have been proposed. Variable results have been obtained by members of a group working simultaneously upon aliquots of the same material, and from time to time different individuals have tested similar materials and have failed to get concordant results.

The reports of Smith<sup>1</sup> indicate that the principal source of error with the alkaline titration method is the difficulty of determining the point of initial turbidity when only small amounts of hydrocyanic acid are present in the solutions. Adverse criticisms have even led to the suggestion that the use of this method should probably be discontinued. Results obtained by Greene (see Smith, 1935<sup>2</sup>), and the findings presented in this report, indicate that such action would be inadvisable without further investigation.

The work presented in this paper was done in collaboration with the Association of Official Agricultural Chemists. The purpose was to ascertain whether a photronic photoelectric turbidimeter<sup>3</sup> was suitable for the determination of small amounts of hydrocyanic acid.

#### METHODS AND MATERIALS

Flaxseed meal furnished by Greene, one of the referees of the Association of Official Agricultural Chemists, and two lots of meal prepared in a similar manner by the writers from flaxseed purchased in the open market were used.

The procedure given by the Association of Official Agricultural Chemists<sup>4</sup> was followed with certain modifications. The distillates were diluted, and 150 ml. aliquots were titrated. Before titration was begun, 1 ml. of a solution of ammoniacal potassium iodide indicator (5 grams of KI+5 ml. of  $\text{NH}_4\text{OH}$ +95 ml. of  $\text{H}_2\text{O}$ ) was added to each aliquot of distillate. Some, but not all distillates were shaken with lead carbonate and filtered before being titrated.

It was thought that some hydrocyanic acid might be lost from the gruel during maceration. To settle this point Guignard's<sup>5</sup> picrate paper was suspended above the macerating gruel in a closed container. The characteristic color change in the paper was soon evident. Apparently hydrocyanic acid vapor had escaped from the macerating gruel. Subsequent to this observation, maceration was carried out in a closed system and precautions were taken to prevent the escape of the vapor during and after maceration. This modification of technic is shown to have increased the amount of hydrocyanic acid recovered.

In one case enough tartaric acid to make a 2.5 per cent solution was added to the gruel just before steam distillation began. In other cases

<sup>1</sup> *This Journal*, 17, 182 (1934); 18, 347 (1935).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Bartholomew and Raby, *Ind. Eng. Chem. Anal. Ed.*, 7, 68 (1935).

<sup>4</sup> *Methods of Analysis*, A.O.A.C., 1930, 287.

<sup>5</sup> *Compt. rend. acad.*, 142, 545 (1906).



definite amounts of liquid hydrocyanic acid of known purity were added to the distillate in order to ascertain whether it could be accurately determined. The liquid hydrocyanic acid was handled in a sealed ampule for accuracy and convenience.

In the first eight and the last two tests maceration occurred at room temperature (20°–25°C.), as directed in the Official Methods. In tests 9–13, inclusive, it occurred at either 23° or 34°C., the temperature being kept constant in an electrically controlled water bath. The latter was done in order to determine whether the temperature variations encountered in the first tests had affected the results.

### RESULTS

Table 1 shows the results of representative sets of determinations. Tests 1 to 8 were on the sample of flaxseed meal supplied by the referee; Tests 9 to 15 were on samples of flaxseed meal prepared by the authors.

TABLE 1.—*Autogenous and added hydrocyanic acid recovered from distillates of flaxseed meal*

| TEST NO. | MACERATING TEMPERATURE | WEIGHT OF SAMPLE | HCN FOUND | HCN ADDED TO DISTILLATE | HCN RECOVERED |
|----------|------------------------|------------------|-----------|-------------------------|---------------|
|          | °C.                    | grams            | mg.       | mg.                     | mg.           |
| 1        | 20                     | 10               | 1.40      | —                       | —             |
| 2        | 23                     | 20               | 2.80      | —                       | —             |
| 3        | 24                     | 10               | 1.40      | —                       | —             |
| 4        | 25                     | 10               | 1.38      | —                       | —             |
| 5        | 25                     | 10               | 1.40      | 5.57                    | 7.05          |
| 6*       | 20                     | 5                | 0.43      | —                       | —             |
| 7†       | 23                     | 10               | 1.62      | —                       | —             |
| 8        | 24                     | 10               | 1.65      | —                       | —             |
| 9        | 23                     | 10               | 1.40      | —                       | —             |
| 10‡      | 23                     | 10               | 1.40      | —                       | —             |
| 11       | 23                     | 10               | 1.29      | —                       | —             |
| 12       | 34                     | 10               | 1.29      | —                       | —             |
| 13       | 34                     | 10               | 1.29      | —                       | —             |
| 14‡      | 22                     | 10               | 1.16      | 4.13                    | 5.29          |
| 15‡      | 23                     | 10               | 1.16      | 4.10                    | 5.26          |

\* Gruel acidified (2.5% tartaric acid added just before distillation).

† Tests 1–8 were made on the same lot of flaxseed meal. In Tests 1–6, maceration occurred in an open distillation flask; in Tests 7 and 8, in the closed distillation system. Tests 9–13, and 14 and 15 were made on meal from two different sources.

‡ Distillate treated with lead carbonate in Tests 10, 14, and 15.

Total calculated values derived from replicate titrations of aliquots of distillates are given in the column "HCN found." The total amounts of autogenous hydrocyanic acid plus liquid hydrocyanic acid added to the distillates are shown under "HCN recovered."

The temperatures (20°–34°C.) at which different macerations took place did not have a measurable effect on the amount of hydrocyanic acid liberated. Maceration in a closed system slightly increased the amount of hydrocyanic acid that could be recovered from the distillate. This may be seen by comparing the results of Tests 1 to 6 with those of Tests 7 and 8 (Table 1). Treating the distillate with lead carbonate had no *visible* effect, but the photoelectric turbidimeter showed that the carbonate had clarified the distillate and increased its light-transmitting quality.

#### DISCUSSION

The results reported appear to justify the continued use of the alkaline titration method inasmuch as large or even very small amounts of hydrocyanic acid can be readily detected with the photoelectric turbidimeter. This not only holds true for this work on flaxseed meal but for the several other kinds of material which, up to the present time, have been tested by the writers with the instrument.

The potassium iodide indicator markedly improved the definition of the end point. This is in agreement with the findings of Sharwood,<sup>1</sup> who showed that this indicator greatly increased the sharpness of the end point for macro determinations.

It has been found in this laboratory and elsewhere (Smith, 1934<sup>2</sup>) that certain volatile organic compounds cause a darkening of the flaxseed meal distillate by the reduction of silver ions. Treating the distillate with lead carbonate before titrating prevented this darkening and permitted the accurate determination of the initial turbidity point.

The results obtained when maceration took place in a 2 per cent tartaric acid solution are in agreement with those of Collins,<sup>3</sup> who found that the action of the glucoside-splitting enzyme was greatly retarded in the presence of even very low concentrations of acid.

No explanation is offered for the slight increase (0.08 mg.) in the amount recovered when liquid hydrocyanic acid was added to the distillate in Test 5 (Table 1).

As shown in Table 1, the differences in amounts of hydrocyanic acid found in different lots of meal from the same source were comparatively large. For example, Tests 9 and 10 differed from Tests 11, 12, and 13 by 0.11 mg. However, replicate titrations of aliquots from any given one of these distillates showed no variation. This held true for all of the distillates reported; the end point for each aliquot of a given distillate was always the same. For this reason it seems that the explanation for the difference between the amounts of hydrocyanic acid that could be found in any two different lots of meal from the same source is related to the process of extraction rather than to the method of determination.

<sup>1</sup> *J. Am. Chem. Soc.*, 19, 400 (1897).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *J. Chem. Soc.*, 102, 586 (1912).

The amounts of hydrocyanic acid reported may appear to be comparatively small. It seems possible that the explanation lies in the fact that the photoelectric turbidimeter detects turbidity before it can be detected with the eye, even when a nephelometric apparatus is used in a darkroom. It was found that after the photoelectric turbidimeter indicated that the end point had been reached, considerably more silver nitrate had to be added before the liquid became visibly turbid.

#### SUMMARY

The photronic photoelectric turbidimeter can be used accurately and rapidly to determine small amounts of hydrocyanic acid distilled from macerated flaxseed meal.

Difference in temperature of maceration within the range 20° to 34°C. had no apparent effect on the amount of hydrocyanic acid extracted.

Hydrocyanic acid was lost by volatilization during maceration when an open distillation flask was used as a macerating vessel.

More hydrocyanic acid was extracted from macerated flaxseed meal by steam distillation than by the use of acid plus steam.

Treating the distillates with lead carbonate prevented the darkening of the solution during titration and permitted a sharper determination of the titration end point.

#### COMPARISON OF METHODS FOR THE DETECTION OF GELATIN IN DAIRY PRODUCTS\*

By CARL S. FERGUSON and PHILEAS A. RACICOT (Massachusetts Department of Public Health, Boston, Mass.)

Certain soured, cultured, fermented or very old dairy products (including sour cream), with or without rennet and not containing gelatin, become cloudy or give distinct precipitates on the addition of picric acid in the Stokes method<sup>1</sup>, now official,<sup>2</sup> for the detection of gelatin. In every case, however, the character of these precipitates differs from that of the precipitate which picric acid produces with gelatin. The gelatin precipitate is finer, more dense, and more likely to remain in suspension, keeping the entire serum cloudy; settles only slowly on standing; and adheres tenaciously to the bottom of the test tube after standing overnight. When present in quantity, it may collect in large sticky lumps which adhere to the test tube on shaking, but it does not flocculate easily on shaking when present in smaller amounts. The precipitates caused by rennet or by decomposition products occurring as a result of fermentation, etc., floccu-

\* Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 1935.

<sup>1</sup> *Analyst*, 22, 320 (1897).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1930, 223.

TABLE 1.—*Results on sour cream by official (Stokes) method*

| APPEARANCE OF—                                     | 1<br>PLAIN   | 2<br>0.25%<br>GELATIN                           | 3<br>0.25%<br>GELATIN<br>AND RENNET | 4<br>0.5%<br>GELATIN | 5<br>0.5%<br>GELATIN<br>AND RENNET | 6<br>PLAIN<br>WITH<br>RENNET          | 7<br>VERY OLD<br>sour cream | 8<br>VERY OLD<br>sour cream<br>WITH 0.5%<br>GELATIN |
|--|--|---|-------------------------------------|----------------------|------------------------------------|---------------------------------------|-----------------------------|---|
| Filtrate from mercuric nitrate treatment           | Clear  | Cloudy  | Cloudy                              | Cloudy               | Slightly turbid                    | Clear                                 | Slightly turbid             | Slightly turbid                                     |
| Picric acid precipitation after shaking            | Clear  | Dense turbidity                                 | Dense turbidity                     | Dense turbidity      | Dense turbidity                    | Heavy rapid-settling ppt. Serum clear | Floc. ppt. soln. clear      | Dense turbidity                                     |
| Picric acid precipitation after standing overnight | Liquor clear, ppt. non-adhesive. Easily rinsed out with cold water | Liquor clear, ppt. tenaciously adhesive to tube | Same as 2                           | Same as 2            | Same as 2                          | Same as 1                             | Same as 1                   | Same as 2   |
| Conclusions  | No gelatin   | Gelatin   | Gelatin                             | Gelatin              | Gelatin                            | No gelatin                            | No gelatin                  | Gelatin   |

late naturally or, if present in small amounts, on shaking. They do not adhere to the test tube, are easily washed out by a stream of water, settle rapidly (leaving the serum practically clear), and on standing agglutinate either at the bottom of the tube or at the surface of the solution. The precipitate produced from rennet rapidly assumes the red color characteristic of Millon's reaction. In the case of mixtures, the gelatin precipitate will remain in suspension long after the flocculent precipitate has settled and, on standing overnight, there will be found the characteristic sticky deposit on the bottom of the test tube.

TABLE 2.—*Results on cottage cheese by official (Stokes) method*  
(Test made on 5 grams of sample plus 10 cc. of water)

| APPEARANCE OF—                                     | 1<br>COTTAGE CHEESE                                 | 2<br>COTTAGE CHEESE +<br>0.5% GELATIN            | 3<br>COTTAGE CHEESE +<br>0.5% GELATIN +<br>RENNET     | 4<br>COTTAGE CHEESE +<br>RENNET<br>NO GELATIN    |
|--|---|--|---|--|
| Filtrate from mercuric nitrate precipitation       | Slightly turbid                                     | Turbid   | Turbid  | Slightly turbid                                  |
| Picric acid precipitation after shaking            | Fine floc. ppt. Liquor clear                        | Dense turbidity                                  | Dense turbidity with heavy, rapid-settling floc. ppt. | Fine floc. ppt. settling in 1 hour. Liquor clear |
| Picric acid precipitation after standing overnight | Liquor clear. Ppt. non-adhesive. Easily rinsed out. | Liquor clear. Ppt. tenaciously adhesive to tube. | Same as 2   | Same as 1  |
| Conclusions  | No gelatin  | Gelatin  | Gelatin   | No gelatin                                       |

Seidenberg<sup>1</sup> attempted to identify the source of the precipitate by further tests on the precipitate itself. Jacobs and Jaffe<sup>2</sup> attempted to remove the interfering substances by use of an adsorbing agent and basic lead compounds as protein precipitants in the preparation of the original filtrate. Richardson and Tarassuk<sup>3</sup> allowed these substances to pass into the original filtrate and then attempted to remove them with trichloroacetic acid, or in the case of any obstinate ones, to prevent their appearance in the final test by using a temperature of 50°C. This seems to prevent the rennet from precipitating but also decreases the sensitivity of the gelatin test because the gelatin precipitate dissolves at this temperature.

<sup>1</sup> *J. Ind. Eng. Chem.*, 5, 927 (1913).

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 4, 418 (1932).

<sup>3</sup> *This Journal*, 17, 314 (1934).

The present study was undertaken in connection with a contested case involving the illegal sale of sour cream containing gelatin, in the examination of which the official Stokes method was applied. The accuracy of this method was contested by the defendant. During the period of the trial this study of the three methods mentioned above was begun.<sup>1</sup> Samples of commercial sour cream as well as of sweet cream that had soured in the laboratory were used. At the time of purchase these samples gave clear solutions on the addition of picric acid as directed in the official Stokes method, showing absence of gelatin. Gelatin in known amounts was then added to some of these samples. Samples of cottage cheese known to contain no gelatin were subsequently prepared for testing by the addition of gelatin or rennet or both. For testing purposes, rennet was also added to some of the sour cream samples. The results from the use of the official method are given in Tables 1 and 2.

#### DISCUSSION

The Jacobs-Jaffe lead nitrate test gave positive results in the absence of gelatin in sour cream and cottage cheese when rennet was present. In the presence of gelatin alone the results were uncertain. With 0.25 per cent gelatin present in sour cream the test was negative, and with 0.5 per cent gelatin the test was only faintly positive (faint cloudiness). It is believed that the adsorbing agent, calcined charcoal, removes some gelatin and results in negative tests when only a small amount of gelatin is present. Such samples would have been positive by the official method.

The Jacobs-Jaffe lead acetate test gave positive results on addition of the tannic acid reagent with sour cream in cases where no gelatin was present and failed to discriminate between rennet and gelatin. The results on the addition of picric acid were indefinite. In both tests tannic acid gave a precipitate or cloudiness with all the sour cream samples tested.

The Richardson-Tarassuk method distinguished gelatin from rennet in sour cream and cottage cheese, but it was less positive than the Stokes test, and an interpretation of the cloudiness was still essential. The sample of cottage cheese without gelatin gave a distinct cloudiness. When cooled to room temperature the test solution became much more cloudy, and heavy precipitates came out in the samples containing rennet.

The trichloroacetic acid does not remove all the interfering substances that give precipitates with picric acid. Heating the test solution to 50°C. causes most of these to remain in solution on adding the picric acid, but in the opinion of the writers some of the gelatin which would precipitate at room temperature is also held in solution. Substances other than gelatin are precipitated by picric acid in the official method, but these can be readily distinguished from a gelatin precipitate by an analyst familiar

<sup>1</sup> This case resulted in conviction and appeal. The defendant subsequently withdrew the appeal and paid the fine of \$200.

with the two types. There seems to be no advantage in any of the methods tried over the official method. Besides being the simplest, the official method appears to be the most accurate method for the detection of gelatin in all types of dairy products. Richardson and Tarassuk confirm this statement as follows:

"The precipitates of gelatin-picrate are more crystalline in nature, settle slowly, and adhere tenaciously to the bottom and sides of the container. The serum will remain opalescent for days. By making use of these observations during the past three years, three separate classes of upper division students were enabled to report correctly on heterogeneously arranged groups of unknown samples of cultured buttermilk."

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## OBSERVATIONS ON THE DETERMINATION OF CUPROUS OXIDE AS APPLIED TO SUGAR ANALYSIS

By R. A. STEGEMAN with D. T. ENGLIS (Department of  
Chemistry, University of Illinois, Urbana, Ill.)

The use of ceric sulfate for the determination of cuprous oxide precipitated by the action of Fehling's or other alkaline copper solutions on reducing sugars has been suggested by the present writers,<sup>1</sup> who outlined a procedure applicable to any determination on pure sugar solutions that did not involve complete reduction of the alkaline copper solution. It was deemed necessary to determine the possible extent of the applicability of the method by a comparison with other volumetric methods, especially those involving the use of dichromate. The speed with which the titration could be completed without loss in accuracy and precision was particularly considered. Since any volumetric method applied to such procedures as that of Munson and Walker<sup>2</sup> would be subject to errors inherent in the original method, as well as to additional errors introduced by the use of a volumetric determination of cuprous oxide, the greatest advantage of a volumetric method would be a saving in time. It was felt that the rapidity with which the determination could be completed should, therefore, be of great importance in making recommendations.

### *I. Gravimetric Methods*

As a basis for comparison, results were referred to those obtained with Munson and Walker's gravimetric method, and by using Soxhlet's formula<sup>3</sup> for the alkaline tartrate and copper solutions. Since the results are expressed in terms of milligrams of cuprous oxide, rather than in

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<sup>1</sup> *Trans. Ill. State Acad. Sci.*, 27, 75 (1934); C.A., 29, 3626.

<sup>2</sup> *J. Am. Chem. Soc.*, 28, 666 (1906).

<sup>3</sup> *J. prakt. Chem.*, 21, (ii), 227 (1878).

terms of reducing sugars, no correction was made for auto-reduction of the Soxhlet reagent.

The asbestos used in all cases, volumetric as well as gravimetric, was ignited and treated as prescribed by Munson and Walker. The weights of crucibles so prepared remained constant or very nearly so, a change of 0.1 and 0.2 mg. being noted in some cases.

Elimination of variables in heating was made nearly complete by use of an electric cone heater. The principal factor affecting the results was time of filtration, which could not at all times be controlled closely, but was found to vary from 30 to 60 seconds. The majority of filtrations were completed in 40 seconds.

As it is generally recognized that the gravimetric method is inaccurate when applied to raw products, the cuprous oxide precipitated was in several cases determined electrolytically by deposition of copper from a sulfuric-nitric acid bath, a current of 2.5 volts and 0.2 amperes being used.

## II. Permanganate Methods

An attempt was made to utilize the official permanganate method,<sup>1</sup> involving the solution of the cuprous oxide in an acidified ferric sulfate solution, followed by titration of the ferrous ion formed with permanganate. This procedure, usually attributed to Bertrand,<sup>2</sup> was reported unsatisfactory by Davis and Daish as early as 1913,<sup>3</sup> but it has been used extensively since, particularly in Europe, presumably because of the apparent ease of operation. Even as late as 1933, in Europe, Baerts suggested the method for the determination of raw sugars.<sup>4</sup>

The method follows:

### REAGENTS

(a) *Potassium permanganate*.—Use approximately 0.1 *N* solutions and standardize in the usual manner against Bureau of Standards sodium oxalate.

(b) *Acid ferric sulfate*.—Dissolve 50 grams of ferric ammonium sulfate in water, add 200 cc. of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), and dilute the solution to 1 liter.

### PROCEDURE

Dissolve the  $\text{Cu}_2\text{O}$  in 50 cc. of acid ferric sulfate solution, titrating as soon as possible after solution with standard  $\text{KMnO}_4$ , beginning the titration slowly, as is customary, but proceeding as rapidly as possible thenceforth to the end point. As the permanganate end point is not easily distinguishable in the titrated solution, continue the titration to the appearance of a greyish color, and estimate the slight excess of permanganate by the addition of sufficient ferrous sulfate to restore the slight greenish color of the cupric-ferric solution. Since the ferric sulfate on standing, especially in the light, may contain appreciable quantities of ferrous ion, run occasional blanks on the ferric sulfate and if necessary make suitable corrections.

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 380.

<sup>2</sup> *Bull. soc. chim.*, 35, (iii), 1285 (1906).

<sup>3</sup> *J. Agr. Sci.*, 5, 437 (1913).

<sup>4</sup> *Sucr. belge*, 52, 186-194 (1933).



## DISCUSSION

In this work, when potassium permanganate was standardized against sodium oxalate, the method proved unreliable, giving results on pure sugar solutions from 3 to 5 mg. lower than those obtained gravimetrically, as shown in Table 1. Somewhat similar results were obtained by Jackson.<sup>1</sup>

TABLE 1.—Comparison of gravimetric and Bertrand methods for estimation of  $\text{Cu}_2\text{O}$ 

| ORDER<br>OF<br>EXPERIMENT | GRAVIMETRIC<br>METHOD | NUMBER<br>OF<br>ANALYSES | BERTRAND<br>METHOD | NUMBER<br>OF<br>ANALYSES | DIFFERENCE |          |
|---------------------------|-----------------------|--------------------------|--------------------|--------------------------|------------|----------|
|                           | mg.                   |                          | mg.                |                          | mg.        | per cent |
| 3                         | 53.1                  | 3                        | 52.0               | 4                        | 1.1        | 2.26     |
| 4                         | 56.2                  | 3                        | 53.2               | 2                        | 3.0        | 5.34     |
| 8                         | 82.4                  | 4                        | 79.2               | 4                        | 3.2        | 3.89     |
| 6                         | 88.4                  | 3                        | 86.2               | 4                        | 2.2        | 2.49     |
| 7                         | 118.8                 | 4                        | 115.3              | 6                        | 3.5        | 2.94     |
| 2                         | 135.4                 | 5                        | 132.5              | 6                        | 2.9        | 2.14     |
| 10                        | 156.0                 | 4                        | 152.6              | 5                        | 3.4        | 2.18     |
| 11                        | 161.5                 | 3                        | 156.7              | 3                        | 4.8        | 2.97     |
| 9                         | 185.4                 | 4                        | 181.0              | 4                        | 4.4        | 2.37     |
| 1                         | 231.5                 | 3                        | 226.6              | 8                        | 4.9        | 2.12     |
| 5                         | 240.5                 | 3                        | 235.7              | 4                        | 4.8        | 1.99     |

Nearly all investigators report that standardization against sodium oxalate leads to low results. It was quite evident from the varying percentage, however, that the difficulty was not entirely one of standardization. Additional data supporting this statement were found in Jackson's report, as well as in the work of Thomas and Dutcher,<sup>2</sup> who reported errors of 8 to 20 per cent. It is the belief of the writers that the error lay in the incomplete solution of the cuprous oxide, as has been suggested by Sullivan<sup>3</sup> and by Kraybill, Youden and Sullivan,<sup>4</sup> or in the atmospheric oxidation of the ferrous ion before and during the titration, this oxidation being catalyzed by the presence of copper.<sup>5</sup> Sullivan showed that good results were obtained if care was taken to insure complete solution by pressing out each visible oxide particle with a glass rod, but the results of some of the collaborators were somewhat lower. This suggests that neither explanation alone is adequate, but that the two are quite inseparable. The extent of atmospheric oxidation would depend on the time consumed in dissolving the cuprous oxide.

Kraybill, Youden and Sullivan stress the necessity for obtaining the correct end point and use an electrometric method. In the work reported here, the application of ortho-phenanthroline ferrous complex, also sug-

<sup>1</sup> *This Journal*, 17, 293 (1934).

<sup>2</sup> *J. Am. Chem. Soc.*, 46, 1662-1669 (1924).

<sup>3</sup> *This Journal*, 18, 382-386 (1935).

<sup>4</sup> *Ibid.*, 19, 125 (1936).

<sup>5</sup> *Poenjak, Am. Inst. Mining Met. Engrs.*, No. 1615D, 10 pp. (1926)

gested by Zerban<sup>1</sup> and by Sullivan,<sup>2</sup> made the end point more distinct, but contributed nothing toward elimination of unsatisfactory results, as indicated in Table 2. Ortho-phenanthroline has proved satisfactory for permanganate titrations,<sup>3,4</sup> and can be applied to this determination if the acidity is kept sufficiently high. Substitution of dichromate for permanganate in the above titration also indicates that the difficulty is not entirely one of standardization nor of incorrect selection of the end point.

TABLE 2.—*Comparison of different oxidizing agents and indicators for titration of ferrous iron formed by solution of Cu<sub>2</sub>O in acid ferric sulfate*  
(Mg. of Cu<sub>2</sub>O indicated)

| GRAVIMETRIC | PERMANGANATE | PERMANGANATE<br>o-PHENANTHROLINE | DICHROMATE<br>BARIUM DIPHENYLAMINE<br>SULFONATE | DICHROMATE<br>o-PHENAN-<br>THROLINE |
|-------------|--------------|----------------------------------|---|-------------------------------------|
| 161.2       | 155.5        | 155.3                            | 155.7   | 155.3                               |
| 161.4       | 155.6        | 155.4                            | 156.4   | 157.5                               |
| 162.0       | 159.1        | 155.7                            | 157.8   | 157.9                               |

The use of heat to insure complete solution of the cuprous oxide without oxidation of the ferrous ion seems applicable if the procedure is conducted in an inert atmosphere, but such conditions seem inadvisable. The error introduced by heating is probably not great, but care must be taken to keep the temperature below 80°C. if ortho-phenanthroline is used as indicator, since it is rapidly destroyed at this temperature. The use of a neutral ferric sulfate solution, acidified only as the titration was carried out, also proved unsuccessful, as solution of the cuprous oxide proved incomplete in all cases. This modification, however, has been noted as the only desirable permanganate method by Kolthoff,<sup>5</sup> who also states that the cuprous ion is oxidized by the air during the reaction with the ferric ion if an acid ferric sulfate solution is used.

### III. Dichromate Method

The use of potassium dichromate was suggested by Jackson and Mathews,<sup>6,7</sup> whose original method required an electrometric determination of the end point in the titration of excess dichromate. The accuracy and precision of this method when applied to pure sugars have been satisfactorily demonstrated by Jackson and Mathews,<sup>7</sup> and have been shown by Jackson and MacDonald<sup>8</sup> to be unaffected by the introduction of o-phen-

<sup>1</sup> Private communication (1934).

<sup>2</sup> *Loc. cit.*

<sup>3</sup> Walden, Hammett and Chapman, *J. Am. Chem. Soc.*, 55, 2649 (1933).

<sup>4</sup> Smith, G. F., Ortho-Phenanthroline, Columbus, Ohio (1934).

<sup>5</sup> Volumetric Analysis, Vol. II, p. 320.

<sup>6</sup> *This Journal*, 15, 199 (1932).

<sup>7</sup> *Bur. Standards J. Research*, 8, 425 (1932).

<sup>8</sup> *This Journal*, 18, 172 (1935).

anthroline ferrous complex as indicator. In the use of *o*-phenanthroline, however, the acidity of the solution must be about 2 *M* at the end of the titration.

Taran<sup>1</sup> suggested diphenylamine for use with dichromate in sugar work. Its disadvantage lies in its variable sensitivity, the color change being sharpest when the titration is carried nearly to completion before addition of the indicator and in the fact that the change from the reduced to the oxidized form is the only one which has been applied with any success. This is probably responsible for the solution of cuprous oxide in acid ferric sulfate and titration of the ferrous ion formed with dichromate.

Sarver and Kolthoff<sup>2</sup> found that barium diphenylamine sulfonate could be substituted for *o*-phenanthroline, and that this indicator overcomes some of the disadvantages of diphenylamine in sulfuric acid. It possesses the advantage over *o*-phenanthroline of producing a more marked color change at the end point, the change from deep purple to light green being more easily discernible in the highly colored solution than is the change in the *o*-phenanthroline complex. It has the disadvantage of requiring an indicator correction, which, however, is small and constant if the volume of the titrated solution is not varied too much. It also requires the presence of phosphoric acid for the best development of its color in dichromate titrations. In addition, the reaction between the indicator and the titrating solution, although rapid, is not instantaneous, and the titration must be carried out more slowly toward the end. However, the approach of the end point can be gaged with little experience by the appearance of the titrated solution, the purple of the indicator reaching its greatest intensity and purity of color just before the color change. This indicator was used in all dichromate titrations except where otherwise noted. This method follows.

#### REAGENTS

(a) *Potassium dichromate*.—Prepare by direct weight, using Baker's A. C. S. reagent. (This has been shown to compare favorably with any other method of standardization,<sup>3</sup> but each solution used was checked by standardization against iron to insure the accuracy of the direct weight method.)

(b) *Ferrous sulfate*.—Make an approximately 0.1 *N* solution by dissolving 39.214 grams of ferrous ammonium sulfate (Mohr's salt) in water, adding 20 cc. of 18 *N* H<sub>2</sub>SO<sub>4</sub>, and diluting to 1 liter. Unless the solution is preserved in an inert atmosphere, make daily comparisons with the dichromate.

(c) *Sulfuric-phosphoric acid*.—To 150 cc. of H<sub>2</sub>SO<sub>4</sub> (sp. gr. 1.84) add 150 cc. of H<sub>3</sub>PO<sub>4</sub> (85%, sirupy) per liter.

(d) *Barium diphenylamine sulfonate*.—0.005 *M* aqueous solution, containing 3.17 grams per liter.

#### PROCEDURE

Separate the Cu<sub>2</sub>O from the excess Fehling's solution as usual. Transfer the mat to the original reaction beaker, adding about 50 cc. of water and disintegrating the

<sup>1</sup> *J. Applied Chem., U.S.S.R.*, 7, 213 (1934).

<sup>2</sup> *J. Am. Chem. Soc.*, 53, 2902 (1931).

<sup>3</sup> Willard and Young, *Ind. Eng. Chem. Anal. Ed.* 7, 57 (1935).

mat thoroughly. Add an excess of standard dichromate solution, acidify, and dissolve the  $\text{Cu}_2\text{O}$ , washing out the crucibles in the acidified solution to remove all  $\text{Cu}_2\text{O}$  adhering to the sides of the crucible. After solution of the  $\text{Cu}_2\text{O}$ , add 15 cc. of the sulfuric-phosphoric acid mixture, dilute with water to 150–200 cc. (if necessary), and titrate the excess dichromate with the ferrous sulfate, using 0.3 cc. of the barium diphenylamine sulfonate as indicator. The color change at the end point is from purple to green. An indicator correction of 0.095 cc. of 0.1 *N* dichromate solution must be subtracted from the total amount used.

### DISCUSSION

Some difficulty was experienced in solution of the cuprous oxide. This was generally true of those particles enmeshed in the asbestos or remaining on the walls of the crucible. Their disintegration by careful and thorough manipulation of the stirring rod delayed the titration. It was believed the use of an acidified dichromate solution might alleviate this difficulty. It has been reported by Kolthoff<sup>1</sup> that acidified dichromate solutions are stable, even on boiling, and checking of the normality over a period of several months indicated the validity of this report.

TABLE 3.—*Effect of gradual addition of acid-oxidizing agents*  
(Data expressed in mg of  $\text{Cu}_2\text{O}$ )

| GRAVIMETRIC | NUMBER<br>OF<br>ANALYSES | DICHROMATE | NUMBER<br>OF<br>ANALYSES | CERIC<br>SULFATE | NUMBER<br>OF<br>ANALYSES |
|-------------|--------------------------|------------|--------------------------|------------------|--------------------------|
| 95.2        | 3                        | 93.4       | 3                        |                  |                          |
| 127.8       | 8                        | 125.0      | 8                        |                  |                          |
| 132.6       | 4                        | 127.7      | 4                        | 127.1            | 4                        |
| 148.7       | 4                        | 146.1      | 4                        |                  |                          |
| 168.4       | 3                        | 164.3      | 3                        |                  |                          |
| 198.0       | 4                        | 193.8      | 4                        |                  |                          |
| 199.6       | 8                        | 197.3      | 8                        |                  |                          |
| 214.1       | 4                        | 206.7      | 4                        | 207.8            | 4                        |
| 304.9       | 8                        | 298.9      | 8                        |                  |                          |
| 362.4       | 4                        | 349.5      | 4                        | 352.7            | 4                        |
| 384.8       | 4                        | 372.0      | 4                        | 374.2            | 4                        |

In the use of acid dichromate, however, it was found necessary to suspend and disintegrate the asbestos mat in a previously measured excess of dichromate. Gradual addition of the acidified solution from a buret or pipet to the cuprous oxide suspension in water, which it was believed might result in a more rapid solution, particularly of the cuprous oxide remaining in the crucible, gave results considerably low, as did ceric sulfate when used similarly (Table 3). Presumably this was due to solution of the cuprous oxide in the acid before sufficient oxidant was added, with subsequent atmospheric oxidation.

<sup>1</sup> *Loc. cit.*, p. 485.

Efforts to speed up the titration by using a previously measured excess of acid dichromate and completing it within 6–10 minutes after removal of the reaction beaker from the source of heat gave results indicative of incomplete solution of the cuprous oxide, the average variation from the gravimetric results of analyses completed within this time being about 0.8 per cent. There appears, therefore, little reason for the use of acidified dichromate solutions. Results with neutral solutions were practically identical if time was given in both cases for complete solution of the cuprous oxide, as indicated in Table 4. Analyses were finished 16–18 minutes after completion of reduction.

TABLE 4.—*Comparison of acid and neutral dichromate used in excess*  
(Mg. of  $\text{Cu}_2\text{O}$  indicated, 8 analyses by each method)

| GRAVIMETRIC | NEUTRAL | ACID  |
|-------------|---------|-------|
| 127.8       | 127.4   | 127.0 |
| 199.6       | 200.5   | 199.8 |

#### IV. Ceric Sulfate

The ceric sulfate method originally suggested called for solution of the precipitated cuprous oxide in an excess of standard ceric sulfate solution (N/2 in sulfuric acid), titration of the excess ceric sulfate with ferrous sulfate, and the use of ortho-phenanthroline ferrous complex as indicator. A color change from blue-green to red-orange denotes the end point. To obviate the necessity for complete transfer of the precipitate, the titration was carried out in the reaction beaker. This modification follows.

##### REAGENTS

(a) *Ceric sulfate*.—Moisten enough ceric sulfate or ceric ammonium sulfate to make a liter of approximately 0.1 *N* solution with 56 cc. of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84). (NOTE: The amount of ceric salt used will vary with the purity of the compound obtained; some statement regarding the purity will usually be found on the bottle. Directions for preparing ceric sulfate can be found in the publications of the G. F. Smith Chemical Company, Columbus, Ohio, who also supply ceric compounds and the indicators mentioned in this paper.) For standardization, it is recommended that an excess of ceric sulfate be added to Bureau of Standards sodium oxalate, the excess being titrated with ferrous sulfate; that the ratio between the two solutions be previously determined; and that ortho-phenanthroline ferrous complex be used as indicator.

(b) *Ortho-phenanthroline ferrous complex*.—Dissolve 6.95 grams of ferrous sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) or an equivalent amount of ferrous ammonium sulfate in water and make up to 1 liter. To this add 14.85 grams of ortho-phenanthroline monohydrate, stirring until all is dissolved and forms a deep red solution.

##### PROCEDURE

Proceed as directed for the dichromate method, except to substitute ceric sulfate, and use 1 drop of ortho-phenanthroline ferrous complex as indicator. Additional

dilute sulfuric acid or hydrochloric acid may be added to increase the acidity and aid in the solution of the cuprous oxide. Phosphoric acid must not be used.

### DISCUSSION

While the method gave reliable results when adequate precautions were taken to insure complete solution of the cuprous oxide, it was found that speeding up the titration gave low and variable results. Heat cannot be applied if ortho-phenanthroline is to be used as indicator, unless the solution be cooled before addition of the indicator and titration of the excess ceric sulfate, which was not deemed practicable. Other indicators tried, including erio-glaucline, erio-green, methyl red in 6 *N* sulfuric acid and diphenylamine in concentrated sulfuric acid, appeared to offer no advantages, all exhibiting variations in sensitivity or unsatisfactory color changes. Ceric sulfate is stable in normal sulfuric acid solutions, and it was found that increasing the sulfuric acid content from 0.5 *N* to normal made solution more complete, but not uniformly so, as the deviation between duplicate analyses was not improved. Specimen results are recorded in Table 5.

TABLE 5.—*Effect of increased H<sub>2</sub>SO<sub>4</sub> content of Ce(SO<sub>4</sub>)<sub>2</sub> solution*  
(Mg. of Cu<sub>2</sub>O indicated)

| GRAVIMETRIC | NUMBER<br>OF<br>ANALYSES | Ce(SO <sub>4</sub> ) <sub>2</sub><br>N/2<br>H <sub>2</sub> SO <sub>4</sub> | NUMBER<br>OF<br>ANALYSES | AVERAGE<br>DEVIATION | Ce(SO <sub>4</sub> ) <sub>2</sub><br>N<br>H <sub>2</sub> SO <sub>4</sub> | NUMBER<br>OF<br>ANALYSES | AVERAGE<br>DEVIATION |
|-------------|--------------------------|--|--------------------------|----------------------|--|--------------------------|----------------------|
|             |                          |  |                          | <i>mg.</i>           |  |                          | <i>mg.</i>           |
| 114.0       | 3                        | 113.7  | 3                        | 0.3                  | 114.2  | 4                        | 0.5                  |
| 161.5       | 3                        | 155.9  | 4                        | 0.5                  | 159.2  | 4                        | 0.3                  |
| 193.0       | 3                        | 187.9  | 4                        | 0.9                  | 192.5  | 5                        | 1.4                  |

The addition of hydrochloric acid has been suggested as an aid to the solution of the cuprous oxide; it does not interfere with the titration in any way, and may serve to sharpen the end point. Even with its use, however, attempts to increase the rapidity of the determination were futile.

In studying the effect on the cuprous oxide produced by the action of reducing sugars on alkaline copper solutions, the writers thought that a careful measurement of a standardized copper solution, followed by complete reduction to cuprous oxide, might make possible an exact comparison of the experimental and theoretical values. However, an effort to extend the method to the determination of cuprous oxide precipitated by complete reduction of Soxhlet's solution was unsuccessful. A similar observation was noted by Jackson and Mathews in their effort to establish the accuracy of their dichromate method.<sup>1</sup> When Ost's solution,<sup>2</sup>

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Chem. Ztg.*, 19, 1784 (1929).

containing carbonate-bicarbonate, was used and only a slight excess of sugar was present, results obtained were within the limits of experimental error. With Soxhlet's solution, no matter how small the amount of sugar in excess, results were quite variable, and the errors were great and far from constant. This was also the case when a large excess of sugar was used in precipitating the cuprous oxide from Ost's solution. These results indicate that the excess sugar interferes to some extent with the determination.

Lejeune<sup>1</sup> has already shown that reducing sugars exert a reducing effect upon ceric salts. However, in view of the results with Soxhlet's solution, it seems quite probable that the excess sugar had less effect upon the determination than did the decomposition products of the partially oxidized sugar. Attention was directed to the asbestos and the cuprous oxide as possible adsorbents. The following experiments pointed toward adsorption on the cuprous oxide: First, dextrose solutions were boiled with either Soxhlet's alkaline tartrate mixture or Ost's carbonate-bicarbonate mixture until the decomposition of some of the sugar was apparent. The solution was then filtered through asbestos, the asbestos was suspended in a measured amount of ceric sulfate, and the ceric sulfate was titrated with ferrous sulfate. Results indicated little or no effect of adsorbed substances on the ceric sulfate solution. Second, a known amount of cuprous oxide, precipitated from Soxhlet's solution or Ost's solution in the presence of a large excess of dextrose, could be determined quite closely if the precipitate was washed previously with cupric sulfate. Some results obtained by this procedure agreed within 3 to 6 per cent of theoretical, this error being within the range occasioned by possible action of any adsorbed sugar or partially oxidized organic matter on the copper sulfate wash solution. A plausible explanation of this would be the fact that tartrates, and apparently the organic products of the sugar oxidation, tend to form complexes with cupric copper, as noted by Shaffer and Hartmann.<sup>2</sup> Inasmuch as copper complexes are usually quite stable, it may be assumed that those formed in the Soxhlet's solution are also quite stable, in which case the requirements for adsorption would probably not be fulfilled under the conditions of the determination involving an excess of cupric copper.

Comparison of the ceric sulfate and dichromate methods on determinations of cuprous oxide precipitated from extremely raw products, such as unclarified chicory and artichoke extracts, in order to magnify any differences in the procedures, indicated that ceric sulfate is affected to a greater extent by impurities than is dichromate, as would be expected from its relative oxidizing power. The results of all determinations, gravimetric as well as volumetric, are, of course, seriously in error when these methods are applied to such products. In addition, the treatment of such

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<sup>1</sup> *Compt. rend.*, 196, 772-773 (1933).

<sup>2</sup> *J. Biol. Chem.*, 45, 349-390 (1920).

solutions previous to analysis causes widely variable results. In some cases, the effect of the volumetric methods was apparently not so great as that of the gravimetric.

#### SUMMARY AND CONCLUSIONS

A study was made of the accuracy and precision attainable in the rapid determination of cuprous oxide precipitated by the action of reducing sugars on Fehling's solution. As a basis for comparison, the volumetric methods used were compared with Munson and Walker's gravimetric method. Particular attention was given to rapidity of solution of the oxide, to its titration with various standard oxidizing agents, and to the use of some of the more recently proposed indicators.

Dichromate and ceric sulfate proved satisfactory when sufficient time was allowed for complete solution of the cuprous oxide in the oxidizing agent. Direct solution of the cuprous oxide in the oxidant eliminated the possible error by atmospheric oxidation encountered in other procedures.

The end point in the permanganate titration was sharpened by the use of ortho-phenanthroline ferrous complex as indicator, but permanganate can not be recommended as preferable to dichromate, although recent work has shown that with care somewhat comparable results may be obtained. It is shown that the standardization of the permanganate against sodium oxalate is not the principal source of error.

Conclusions are based on the rapidity of the analyses, as well as on the accuracy.

The use of barium diphenylamine sulfonate or ortho-phenanthroline ferrous complex as indicator for the dichromate method is suggested. The present high cost of ceric sulfate and the easy preparation of standard dichromate solutions can not be overlooked.

Attempts to establish the accuracy of the cuprous oxide estimation by complete reduction of a standard Fehling's solution were unsatisfactory. A possible explanation for the failure is suggested.

Preliminary work on raw sugars indicates that dichromate is less affected than are the other oxidizing agents, although, as might be expected, none of the oxidation methods can be successfully applied to very impure solutions.

#### DETERMINATION OF IRON IN BEER

By WM. SIEBENBERG and W. S. HUBBARD (Schwarz Laboratories, Inc., New York, N. Y.)

Beer is very sensitive to metals. Because as little as 1 p.p.m. of iron may affect its clarity, it is necessary to have a method for its accurate determination. Beer normally contains approximately 0.5 p.p.m. of iron, but many samples contain much less.



A colorimetric method for the determination of iron quite commonly used is that of Andrew Thomson.<sup>1</sup> This method has been adapted to beers except that ferrocyanide<sup>2</sup> is used instead of thiocyanate. The writers claim that the method is sensitive to 1 part of iron in 1,500,000 parts of water. However, they prefer an ashing procedure to a wet digestion. When wet digestion is used, the beer will froth badly and often digest poorly, owing to the large quantities necessary to use to obtain sufficient iron for an accurate determination. Ferrocyanide was adopted as the reagent, since it is readily obtained by all chemists and gives uniformly accurate results. Lyons suggests thioglycollic acid<sup>3</sup> in place of ferrocyanide, but this acid is not so easily obtainable and its odor is objectionable in a brewing laboratory. Beer is very sensitive to odors and easily and quickly absorbs them.

Beers ordinarily contain traces of copper, and sometimes zinc and lead as well as iron. With the use of nickel tanks in the brewery and the packaging of beer in tin cans, nickel and tin have also been found. Therefore it was considered to be necessary to devise a method that would eliminate these various metals and interfering substances before a quantitative determination of iron could be made. The method that has been studied in these laboratories for several years and that has been refined to take care of nickel and tin is as follows:

#### METHOD

Open the bottle or can of beer and transfer part of the contents to a dry acid-washed container of sufficient capacity to permit easy and rapid decarbonating; shake the bottle or can and transfer the balance of the beer to the container. Precaution should be taken to obtain a uniform sample because any sediment or slight precipitate in a beer may contain part of the iron, especially when large quantities are present. After the sample has been decarbonated sufficiently, transfer 150 cc. of the fully representative sample to a silica dish, which previously has been acid-washed and ignited. Place the dish on a hot plate and evaporate the contents to approximate dryness and char slightly. Continue charring by the use of a small flame, adjusting it so that the dish does not become dull red. When the contents appear completely charred, carefully invert the black mass with a platinum rod and continue charring until gases no longer are emitted. Place the dish in a muffle held between 500°–550° C. (not over 550°),<sup>4,5</sup> and maintain at that temperature until the ash is white. Cool, and slowly and cautiously add 25 cc. of HCl (1+1). Allow the dish to stand on a steaming water bath for at least 20 minutes.

Quantitatively transfer the entire contents to an acid-washed beaker. When somewhat cool, carefully neutralize the solution with  $\text{NH}_4\text{OH}$ , using two drops of methyl orange indicator. Make the solution approximately 0.25  $N^6$  with HCl, using 2 cc. of concentrated U.S.P. HCl for every 100 cc. of solution. (At the time of acidification, the volume of the solution should be between 150 and 200 cc.) Cool carefully and run in  $\text{H}_2\text{S}$  until completely saturated (about 10–15 minutes). Allow the

<sup>1</sup> Sutton, *A Systematic Handbook of Volumetric Analysis*, p. 244 (1924).

<sup>2</sup> *Quantitative Chemical Analysis*, p. 265 (1933).

<sup>3</sup> *J. Am. Chem. Soc.*, 49, 1916 (1927).

<sup>4</sup> Wichmann, Murray, Harris, Clifford, Loughrey and Vorhes, *This Journal*, 17, 115 (1934).

<sup>5</sup> Gebhardt and Sommer, *Ind. Eng. Chem. Anal. Ed.*, 3, 24 (1931).

<sup>6</sup> McAlpine and Soule, *Qualitative Chemical Analysis*, p. 265 (1933).

solution to stand 30 minutes, and filter through a quantitative filter (Whatman No. 41). When the entire solution has drained, wash three times with a saturated water solution of  $\text{H}_2\text{S}$ . Allow the filter to drain completely after each washing. (Copper, lead, and tin are left on the filter, while iron and nickel will be found in the filtrate.) Combine the filtrate and washings and boil off the excess  $\text{H}_2\text{S}$ , testing from time to time with lead acetate paper. When free of  $\text{H}_2\text{S}$ , add an excess of bromine water to oxidize the reduced iron. Continue the boiling until the excess bromine has been removed and the entire solution has been reduced to 50-75 cc. Neutralize the excess acid with ammonia and make distinctly acid with 1 to 2 drops of  $\text{HCl}$ . Cool the mixture in an ice-bath, and add cold distilled water to bring the volume in the beaker to 100 cc. Buffer the solution by the addition of 10 cc. of a 50% solution of ammonium acetate, followed by 0.5 cc. of glacial acetic acid.<sup>1</sup> Stir well and filter while cold through a quantitative paper (Whatman No. 42). When the solution has drained completely, wash the beaker and filter several times with *cold* distilled water, draining each time before washing. The ash of beer contains approximately 45%  $\text{P}_2\text{O}_5$ , which is enough to precipitate all iron as phosphates. Any nickel will remain in the filtrate, which may be used for a quantitative determination (one precipitation is sufficient). In the precipitation beaker, mix 10 cc. of concentrated  $\text{HCl}$  with 40 cc. of water, heat to boiling, and immediately use this solution to dissolve the iron precipitate on the filter paper, collecting the filtrate<sup>2</sup> and washings in a 100-200 cc. volumetric flask, depending upon the amount of iron present. Cool, and make up to mark.

Take a volume of the solution representing not more than 0.1 mg. of Fe (usually 10-20 cc.) and place in a 100 cc. Nessler tube (or in a large test tube). In similar tubes prepare standards of 0.02, 0.04, 0.06, 0.08, 0.10, and 0.12 mg. of Fe. Add 1 cc. of concentrated  $\text{HCl}$  and make up all the solutions to the same volume (level), using distilled  $\text{H}_2\text{O}$  (about 20 cc.). To each tube add 1 cc. of the 10% ferrocyanide solution, let stand for 30 minutes, and compare the colors. It is important that the standards and the sample be run at the same time and treated in the same manner, both with regard to the addition of ferrocyanide solution and to the concentration of  $\text{HCl}$ . The standard solution of iron (ferric) may be prepared as directed by Sutton.<sup>3</sup>

In order to determine how this method might work in a synthetic mixture similar to beer, a solution was prepared containing the usual amounts of inorganic salts, sugars, dextrans, and protein found in beer. Iron determinations were made on each of the ingredients used, and from the figure obtained the amount of iron theoretically present in the solution was calculated. The iron in solution was estimated to be 0.55 p.p.m., a figure approximate to that actually found in most beers.

Definite quantities of an iron salt were added to different portions of the synthetic beer and these were pasteurized. Three analysts who examined these solutions had no knowledge of the iron content of the samples which they received. The results are shown in Table 1.

A second set of samples analyzed contained only the quantities of inorganic salts usually found in beer. The amount of iron in this solution was calculated to be 0.0045 p.p.m., which figure is negligible. The results are shown in Table 2.

<sup>1</sup> Lawrence, *J. Ind. Hyg*, 8, 12 (1926).

<sup>2</sup> *Loc cit*

TABLE 1.—*Synthetic solution*

| SAMPLE NO. | IRON (Fe) FOUND | IRON (Fe) ADDED+BLANK | DEVIATION |
|------------|-----------------|-----------------------|-----------|
|            | <i>p.p.m.</i>   | <i>p.p.m.</i>         |           |
| 1          | 2.00            | 1.99                  | +.01      |
| 2          | 2.50            | 2.53                  | -.03      |
| 3          | 2.10            | 1.99                  | +.11      |
| 4          | 2.33            | 2.35                  | -.02      |
| 5          | 3.00            | 3.07                  | -.07      |
| 6          | 0.83            | 0.55                  | +.28      |
| 7          | 0.00            | 0.55                  | -.55*     |
| 8          | 3.00            | 2.71                  | +.29      |
| 9          | 1.50            | 0.91                  | +.59*     |

\* Omitted from deviation average. Average deviation: 0.115 p.p.m.

TABLE 2.—*Synthetic solution*

| SAMPLE NO. | IRON (Fe) FOUND | IRON (Fe) ADDED | DEVIATION |
|------------|-----------------|-----------------|-----------|
|            | <i>p.p.m.</i>   | <i>p.p.m.</i>   |           |
| 1          | 1.00            | 0.84            | +.16      |
| 2          | 0.90            | 0.42            | +.48      |
| 3          | 1.67            | 1.68            | -.01      |
| 4          | 0.33            | 0.00            | +.33      |
| 5          | 3.17            | 3.36            | -.19      |
| 6          | 0.25            | 0.00            | +.25      |
| 7          | 3.36            | 3.78            | -.42      |

Average deviation: 0.26 p.p.m.

TABLE 3.—*Real beer*

| SAMPLE NO. | IRON (Fe) FOUND | ADDED IRON (Fe) | DEVIATION |
|------------|-----------------|-----------------|-----------|
|            | <i>p.p.m.</i>   | <i>p.p.m.</i>   |           |
| 1          | 0.20            | 0.00            | +.20      |
| 2          | 4.92            | 4.20            | +.72*     |
| 3          | 1.64            | 1.68            | -.04      |
| 4          | 1.23            | 1.26            | -.03      |
| 5          | 0.82            | 0.84            | -.02      |
| 6          | 4.00            | 4.20            | -.20      |
| 7          | 1.00            | 0.84            | +.16      |
| 8          | 0.00            | 0.00            | +.00      |
| 9          | 1.43            | 1.68            | -.25      |
| 10         | 1.27            | 1.26            | +.01      |

\* Omitted from deviation average. Average deviation: 0.10 p.p.m.

TABLE 4.—*Real beer*

| SAMPLE NO | IRON (Fe) FOUND | ADDED IRON (Fe) | IRON (Fe) FOUND<br>(CORRECTING FOR BLANK) | DEVIATION |
|-----------|-----------------|-----------------|---|-----------|
|           | <i>p.p.m.</i>   | <i>p.p.m.</i>   | <i>p.p.m.</i>                             |           |
| 1         | 1.50            | 0.00            | 0.00                                      | + .00     |
| 2         | 1.83            | 0.42            | 0.33                                      | - .09     |
| 3         | 2.83            | 1.26            | 1.33                                      | + .07     |
| 4         | 3.50            | 2.10            | 2.00                                      | - .10     |
| 5         | 5.33            | 3.78            | 3.83                                      | + .05     |

Average deviation · 0.06 p.p.m.

Two lots of bottle beer were then treated with a definite amount of the iron salt. One beer was selected because it was practically free of iron (one analyst found none and another analyst found 0.20 p.p.m.); the other beer selected contained 1.50 p.p.m. as determined by one analyst. The resulting figures are shown in Tables 3 and 4.

#### DISCUSSION

It will be noted that the results on the synthetic beers, Tables 1 and 2, do not check so closely as those obtained on the real beers, Tables 3 and 4. This no doubt is due to the fact that it was rather difficult to keep the synthetic samples in perfect solution and therefore to obtain an entirely representative sample. In order to obviate the chances of any iron being precipitated from the real beer, the iron salt was added directly to the sample measured out for analysis.

The writers wish to express their thanks and appreciation to members of the laboratory staff of Schwarz Laboratories, Inc., particularly to A. A. Jackson, Robert D'Orazio, and Julius Siebenberg, for developing part of the data presented in this paper.

#### A COLORIMETRIC METHOD FOR THE DETECTION OF TEA SEED OIL IN OLIVE OIL

By J. FITELSON (U. S. Food and Drug Administration,  
New York City)

Commercial tea seed oil is expressed from the seeds of a tea plant (*Thea sasanqua*, Nois), which is grown in China, Assam, and Japan for its oil-bearing properties. The kernels contain up to 60 per cent of oil. The seeds of the ordinary tea plant (*Thea sinensis*) contain from 16 to 26 per cent of oil in the kernels. Practically all the crude tea seed oil used in the United States is imported from China, and is used mainly in the

textile and paint industries. Refined tea seed oil can be used as an edible oil; it does not contain any saponin, although its presence has been suspected.<sup>1</sup>

Owing to the close resemblance to olive oil in its chemical characteristics, tea seed oil has been used as an adulterant for olive oil. The glyceride and fatty acid components of tea seed oil and olive oil are almost identical<sup>2,3</sup> so that the usual chemical analysis does not differentiate these oils. Many color tests have been proposed for the detection of tea seed oil, but all of these methods have been shown to be unreliable.<sup>4,5</sup> The color tests based on treatment with nitric acid<sup>6,7</sup> were also tried in this laboratory on olive oil and refined tea seed oil and proved to be of no value.

It has been claimed by Lewis<sup>8</sup> that tea seed oil differs from olive oil in the absorption of ultraviolet light and also in the fluorescence produced by the decolorized oils in ultraviolet light<sup>9</sup> but investigation on numerous samples shows such great variations in the behavior of these oils that these optical methods cannot be used for the detection of the adulteration of olive oil with tea seed oil.<sup>10</sup>

The titer test has been suggested by Jamieson<sup>11</sup> for the detection of 25 per cent or more of tea seed oil in olive oil. This suggestion is apparently based on a reported range of 13°–14.5°C. for the titer of tea seed oil in contrast to the 17.2°–26.4° C. range for olive oil. It is not stated whether these figures for tea seed oil were obtained from the crude or the refined oil. Uchida<sup>12</sup> obtained a titer of 25.5° C. for tea seed oil. The wide range in the titer of olive oil makes this proposed method of little value because the adulteration of an average olive oil with over 50 per cent of tea seed oil would yield a product with a titer within the recorded limits for pure olive oil.

Recently, the unsaponifiable matter has been studied by a number of investigators in efforts to evolve a method for the detection of tea seed oil in olive oil. Although the phytosteryl acetates from these oils have different melting points, it has been found by Pritzker and Jungkunz<sup>13</sup> that the melting point of the phytosteryl acetate obtained from a mixture of olive oil and tea seed oil coincides with that obtained from pure olive oil. Bolton and Williams<sup>14</sup> proposed the use of the iodine number of

<sup>1</sup> Lewkowitch and Warburton, *Chemical Technology and Analysis of Oils, Fats and Waxes*, Vol. 2, p. 332. London (1922).

<sup>2</sup> Hilditch and Jones, *J. Soc. Chem. Ind.*, 53, 13–21 T (1934).

<sup>3</sup> Griffiths and Hilditch, *Analyst*, 59, 312 (1934).

<sup>4</sup> Caulkin, *Pharm. J.*, 118, 769, (1927).

<sup>5</sup> Pritzker and Jungkunz, *Z. Untersuch. Lebensm.*, 69, 542 (1935).

<sup>6</sup> Allen's Commercial Organic Analysis, 5th ed., Vol. 2, p. 160.

<sup>7</sup> Eldson, *Edible Oils and Fats*, p. 248. London (1926).

<sup>8</sup> *Analyst*, 60, 16 (1935).

<sup>9</sup> Cocking and Crews, *Pharm. J.*, 133, 86 (1934); *Quart. J. Pharm.*, 7, 531 (1934).

<sup>10</sup> Private communication from B. A. Brice, U. S. Food & Drug Adm., Washington, D. C.

<sup>11</sup> *Vegetable Fats and Oils*, p. 145 (1932).

<sup>12</sup> *J. Soc. Chem. Ind.*, 35, 1089 (1916).

<sup>13</sup> *Loc. cit.*

<sup>14</sup> *Analyst*, 55, 5 (1930).

the unsaponifiable matter, but the work of Jamieson and McKinney<sup>1</sup> shows such a wide variation in the iodine numbers of the unsaponifiable matter of olive oils that this method would be of doubtful value for regulatory purposes.

The work that has been done on tea seed oil and olive oil shows that these oils differ mostly in the character of their unsaponifiable matter. The writer, therefore, applied the various sterol color tests to the unsaponifiable matter of olive oil and tea seed oil and modified the Liebermann-Burchard reaction for cholesterol<sup>2</sup> until a specific color reaction was obtained for tea seed oil. In this reaction chloroform, acetic anhydride, and sulfuric acid are added to the unsaponifiable matter of the oil. Tea seed oil produces a deep fluorescent color, green by reflected light and brown by transmitted light. Olive oil and the other common edible vegetable oils show a green color. Occasionally, olive oil gives a faint green-brown fluorescence. On addition of anhydrous ethyl ether at this stage, tea seed oil produces an intense red color, which slowly fades to a light brown. None of the other oils exhibits this deep red color, although some olive oils show a faint tinge of pink before fading to the final light brown. When it was found that these colors could be obtained directly from the oils as well as from the unsaponifiable matter, after suitable adjustment of the concentrations of the reagents, the test was simplified. No color is produced by the free fatty acids from these oils. Anhydrous ethyl ether appears to be the only common organic solvent that causes the appearance of the red stage with tea seed oil, and the quantity present in mixtures made with olive oil can be determined approximately by the intensities of the red color produced. When the test is carried out at room temperature, the red color reaches its maximum intensity in about one minute after addition of the ether, and within one to two minutes begins to fade. This transition can be slowed down by cooling in ice water to permit the stability of the maximum colors for about 5 minutes for comparison with known tea seed oil mixtures tested simultaneously. Table 1 shows the colors obtained from common oils at several stages of the test.

An exhaustive examination of all types of olive oil was made to establish the reliability of this test. Sixty samples of olive oil were obtained from importations of small containers (cans and bottles) immediately after arrival in this country. In addition, 197 samples of olive oil imported in bulk (drums and barrels) were obtained. These imported oils were in the custody of the U. S. Treasury Department at the time of sampling. The following table shows the source of the oils sampled:

| Country of Origin | Spain | France | Algeria | Tunis | Italy | Greece | Syria | Total |
|-------------------|-------|--------|---------|-------|-------|--------|-------|-------|
| No. of Samples    | 147   | 16     | 8       | 11    | 68    | 6      | 1     | 257   |

<sup>1</sup> *Oil and Soap*, 10, 69 (1933)

<sup>2</sup> Matthews, *Physiological Chemistry*, p. 90 (1928).

TABLE 1.—Colors produced by oils in tea seed oil test

| OIL*          | COLOR<br>(BEFORE ADDITION OF ETHER) |                              | COLOR<br>(AFTER ADDITION OF ETHER) |                |                  |
|---------------|-------------------------------------|------------------------------|------------------------------------|----------------|------------------|
|               | AFTER 1 MIN.                        | AFTER 5 MIN.                 | INITIAL                            | CHARACTERISTIC | FINAL            |
| 1. Corn       | deep blue                           | blue-green                   | green                              | green          | green            |
| 2. Peanut     | brown                               | brown-green                  | brown-green                        | brown-green    | brown            |
| 3. Rape       | light green                         | green                        | green                              | green          | green            |
| 4. Sunflower  | deep green                          | green                        | green                              | brown          | light brown      |
| 5. Soya Bean  | red-brown                           | brown-green                  | brown-green                        | brown-green    | brown            |
| 6. Cottonseed | brown                               | brown-green                  | green                              | green          | green            |
| 7. Sesame     | green                               | green                        | green                              | green          | green            |
| 8. Coconut    | light brown                         | light green                  | light green                        | light brown    | light brown      |
| 9. Olive      | light green                         | green                        | green                              | brown          | gray to<br>brown |
| 10. Tea Seed  | deep green                          | deep fluorescent green-brown | brown-green                        | deep red       | light brown      |

\* All refined oils except the olive oil.

These oils represent all grades of olive oil, including "foots" and "extracted" oils. In addition, 14 samples of California olive oil obtained at the factories immediately after pressing, and 13 samples pressed from various types of ripe olives in this laboratory were examined. A total of 284 olive oils was examined, and none of these gave the characteristic tea seed oil colors, i.e. initial fluorescence and final intense red color.

The test was applied to 31 samples of crude tea seed oil and to 24 samples of refined tea seed oil and in every case the characteristic reactions were obtained. Tea seeds (*Thea sasanqua*) were obtained from China, and the oil was extracted and expressed from the kernels. Oil was also expressed and extracted from the seed of the ordinary tea plant (*Thea sinensis*). The characteristic test was obtained from the oil from both of these seeds. The substance producing the color reactions in this test is quite stable; all attempts to destroy it without impairing the edibility of the oil were futile. The red colors produced by the refined tea seed oils were of the same order of intensity, varying within plus or minus 10 per cent of the color given by an average oil.

#### METHOD

For a preliminary qualitative test use the following room temperature method. Measure into a test tube (18×150 mm. is a convenient size) exactly 0.8 cc. of acetic anhydride, 1.5 cc. of chloroform, and 0.2 cc. of concentrated sulfuric acid. Mix, and cool to room temperature. Add 7 drops of the oil to be tested directly to the reagents, mix, and cool again. (To measure the 7 drops of oil use glass tubing, 4 mm. outside diameter and approximately 2 mm. inside diameter. These 7 drops should weigh approximately 0.22 gram.) If the solution of oil in the reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each

addition until a clear solution is suddenly formed. Appreciable deviations from these quantities, particularly in the sulfuric acid, cause distinct variations in color intensities. Since the mixed reagent deteriorates slowly, do not mix in advance of testing.

After the test tube and contents have remained at room temperature for 5 minutes, note the color produced. Tea seed oil will exhibit a deep green by reflected light and brown by transmitted light. Olive oil will show a green color by reflected and transmitted light, occasionally exhibiting a faint fluorescence. Add 10 cc. of anhydrous ethyl ether from a graduated cylinder and mix immediately by inverting once. Tea seed oil will show a brown color changing to an intense red within a minute or so. This red color reaches a maximum and then fades slowly within a period of a few minutes. Olive oil forms an initial green color on addition of the ether. This color fades slowly to a brown-gray, occasionally passing through a faint pink stage. Both olive oil and tea seed oil will eventually fade to a permanent light brown color. Mixtures of tea seed oil and olive oil show the characteristic tea seed oil colors proportional in intensity to the quantity of tea seed oil present.

For approximately quantitative estimations drop the oil into the reagents as described above and allow to remain at room temperature for 5 minutes. In the meantime, cool a 10 cc. portion of anhydrous ethyl ether in ice water. At the end of the 5 minute period, place the test tube containing the oil and reagents in the ice water for 1 minute, add the cold ether (taking care that no water falls into the test tube), and mix. Return the tube to the ice water bath and allow the colors to develop while it is immersed in the ice water. The colors will develop slowly and reach a maximum within 5 minutes. This maximum intensity will remain stable for 5-10 minutes before beginning to fade.

Use the deepest red colors produced as a basis for comparison, and because of the short period of stable maximum intensity it is not convenient to test more than three oils at one time. Standards containing known quantities of tea seed oil in an olive oil that gives little or no pink color with this test, should be run simultaneously with the sample. The preliminary room temperature test will give an indication of the standards to be used in the ice water method.

#### SUMMARY

A color test for the detection of tea seed oil in olive oil is described. This test is specific and characteristic for tea seed oil and is not given by the other common edible vegetable oils.



## BOOK REVIEWS

**Fundamentals of Dairy Science.** By ASSOCIATES OF LORE A. ROGERS. 615 pp. Reinhold Publishing Corporation, New York, 1936. Price \$6.00.

It would be an act of temerity indeed to attempt, single-handed, to review a book of this scope, representing as it does the combined efforts of more than a score of outstanding specialists in the far-flung field of dairy science. The (ostensible) reviewer is indebted to Mr. R. U. Bonnar for the comment on "The Physical Chemistry of Milk and Milk Products" (136 pp.), to Dr. A. C. Hunter for that on "The Microbiology of Milk and Milk Products" (182 pp.), and to Dr. E. M. Nelson for that covering "The Nutritional Value of Milk and Milk Products and the Physiology of Milk Secretion" (148 pp.). Dr. Nelson has also contributed comment on Chapter IV, entitled "Pigments of Milk."

For those already familiar with the earlier edition it need only be said that every chapter bears marks of the most careful revision. A glance at the bibliographies (averaging more than 100 titles to a chapter) discloses many references to work published in the 1930's, and not a few are as late as 1934. All the references are critically selected and have a direct bearing upon the material presented in the text. The numerous excellent tables, graphs, and cuts are also adequately referenced. The general up-to-dateness of the material and the fair manner in which controversial matters have been treated give promise of a realization of the hope of the Editorial Committee that it will "stimulate research along lines now lagging, thereby correcting somewhat the lack of balance in our knowledge of the scientific basis of the dairy industry."

For those unfamiliar with this valuable compilation by past and present associates of Dr. Rogers of the U. S. Bureau of Dairy Industry, it is, perhaps, in order to quote a portion of the preface: "The effort has been made to present basic data, fundamental observations, and unbiased discussions of researches that contribute to the present status of the dairy industry. The matter has been arranged in such a way as to bring together topics considered from the same scientific angle rather than to group them under the more usual headings of specific products of dairy manufacture." Accordingly, one will find the whipping of cream, the foaming of milk, and the churning of butter treated in the chapter on physical equilibria of milk; cheese manufacture under coagulation of milk; ice cream under freezing of milk and milk products, and so on. In like manner the chapter on the Proteins of Milk proceeds logically from the chemistry of casein to its preparation and uses, and the chapter on Lactose is developed in a similar manner. The book is indispensable not only to advanced students and research workers in dairy science—for which it is primarily intended—but to regulatory officials as well. Any confusion which the method of treatment might cause the latter group is largely obviated by the excellent subject index. There is much to be said for the commendable effort to bring theory and practice together in the same chapter, with the ultimate purpose of bringing within speaking distance workers in the fields of pure and applied dairy science.

In the chapter on the Composition of Milk and Milk Products, in the 122 page part entitled "The Constituents of Milk," the authors are to be congratulated on citing data on milk composition which correct the impression given by earlier texts that 13 per cent total solids, 4 per cent fat, and 5 per cent lactose still represent the average composition of American milk. The wisdom of refraining from citing foreign data on milk composition is apparent, although it might, perhaps, have been interesting to cite the studies of Tocher on the correlation between lactose and milk yield. It is perhaps in order at this point to observe that the compilation of data on milk composition, milk fat constants, and the like, based on authentic samples from

relatively small herds of cows, is of limited diagnostic value to regulatory officials. In modern factory practice, fluid milk and other dairy products represent the output of relatively large numbers of cows. It therefore seems reasonable to suppose that the remarkable constancy in citric acid content reported by Hartmann and Hillig (cited on page 22) would be duplicated in the other constituents, and on the fat constants, of commercial fluid milk. The State regulatory official has a splendid opportunity to render service in this field in connection with his normal activities.

The figures cited on page 34 on the composition of evaporated whole milk and sweetened condensed whole milk are, perhaps, the best that the literature affords. However, some doubt may be expressed that anything like these ranges and averages in fat and milk solids will be exhibited today by these closely standardized products. In the discussion on page 88 of the dangers of neutralization of high acid cream for butter manufacture, the question of the utilization of cream of questionable wholesomeness might have been profitably raised. Regulatory officials will be a little startled to see that brash infant, process or emulsified, cheese, masquerading, on page 245, under the time-honored name "loaf cheese."

In the part, "Physical Chemistry of Milk and Milk Products," the monograph depicts the phenomena involved from a physico-chemical view-point. There is a noticeable lack of correlation of the various phenomena. From the conflicting nature of much of the experimentation, this lack of correlation appears justified. It is to be noted that those phenomena of immediate practical use are fully outlined, while those experiments which bear rather upon a knowledge of fundamental phenomena are conflicting. Certainly this part of the book presents an opportunity for enterprise in investigation of actual, rather than ideal, systems.

The treatment of the alkaline titration of milk is a step in the direction of rigid interpretation of such phenomena which may well be further advanced in newer work. Failure to apply thermodynamic reasoning to the temperature functions may well be based either upon the wide variance encountered in practice or upon the lack of sufficient data, and so may be excused. Here is another opportunity for continued study. Probably the phase relations will remain the hardest mystery to penetrate.

Fortunately, the extensive bibliography in the professional literature may furnish investigators the clues necessary to start where this monograph ceases, without painful searches.

Chapter XIV on Nutritional Value of Milk and Milk Products is by far the longest chapter in the book (100 pp.), which is in keeping with the rapid developments and keen interest in the field of nutrition. The subject matter is divided into the nutritional requirements of mammals, the nutritive properties of milk, and the practical aspects of milk and milk products as foods. The authors have succeeded admirably in presenting the subject matter in simple language; this fact, together with the fact that 286 references to the original literature are appended, gives the chapter a wide scope of usefulness. The authors have presented the important aspects of the findings of investigators in the field and have at the same time attempted to evaluate these contributions. This makes the chapter something more than a mere summary of facts.

Chapter XV on Physiology of Milk Secretion deals with a subject that is primarily of scientific interest, although some parts which give consideration to factors which influence milk yield and composition of milk are of practical importance.

Chapter IV on Pigments of Milk is a clear and concise discussion in which the fat-soluble and water-soluble pigments of milk are considered separately. The discussion relating to the fat-soluble pigments deals primarily with carotene, and subject matter is presented relating to its constitution, isolation and determination in

milk and butter, occurrence in milk and feeds, and the color of butter. The only water-soluble pigment covered is lactoflavin (lactochrome), but it is stated that "the possibility exists that more than one representative of the lyochrome class of pigments occurs simultaneously in cow's milk."

The preparation of lactoflavin, its constitution, properties, and significance are discussed. The importance of these constituents of milk from a nutritional standpoint is merely mentioned in this chapter, but discussed at greater length in Chapter XIV.

For those interested in the relationship of basic bacteriological phenomena to dairying and dairy manufacture, the part entitled "The Microbiology of Milk and Milk Products" is an excellent source of information.

In their manner of presenting information the authors have compiled a rather unique contribution to the literature of dairy bacteriology. Each phase of the subject has been approached through a discussion of the fundamental principles of bacteriology and biochemistry involved, immediately followed by an explanation of the application of these principles to dairying practices and to products of dairy manufacture. In such a method of treatment the authors have necessarily devoted a considerable number of pages to the presentation of fundamental information regarding ecology, morphology, bacterial nutrition and metabolism, taxonomy, and the cultural characteristics of micro-organisms. The bringing together of topics involving the same bacteriological, or chemical, principle, rather than a division into chapters, each dealing with a specific commodity, results in the inclusion in a single chapter of discussions of pure, scientific principles, various processes of dairy manufacturing, hygienic handling of milk and milk products, and the causes of defects in dairy products. The entire section on microbiology is divided into four chapters, dealing, respectively, with sources of bacteria, metabolism and growth of bacteria, physical and chemical factors in bacterial growth, and yeasts and molds. Topics such as the spread of disease by dairy products, care in handling milk, the production of butter, the ripening of cheese, refrigeration, and pasteurization, to name a few, are inserted in the appropriate chapters devoted to that basic principle which applies. While this form of presentation is somewhat confusing to the reader searching for information relative to the microbiology of a specific product, the arrangement is logical in the light of the authors' declared intention "to present basic data, fundamental observations and unbiased discussions of researches that contribute to the present status of the dairy industry" and to point out how these data apply in specific instances.—W. B. WHITE.

**American Chamber of Horrors.** By RUTH DE FOREST LAMB. Farrar & Rinehart, Inc., New York, 1936. Price \$2.50.

Although scarcely classable as a scientific work, the presentation of a review in *This Journal* readily finds justification in the fact that, throughout, this book deals with matters intimately concerned with the pursuit of science as fundamentally embodied in the work of the food and drug analyst, the hygienist, and the sanitarian.

Sub-titled "The Truth About Foods and Drugs," and deriving its name from that of the now famous exhibit of representative perils and frauds to which the American consumer is exposed, this book constitutes a detailed and graphic exposition of these, of the manifold activities of the Food and Drug Administration for their correction, and of the serious handicaps by which the latter is at present confronted; all of which leads up to and strikingly sets forth the urgent need of a radical revamping of the now obsolescent Food and Drugs Act of a generation ago.

Shocking though many of the revelations must be to the general public, and making due allowance for the sensational and seemingly exaggerative style of ex-

pression, those of us on the inside know that the story is an accurate portrayal of conditions as actually uncovered. Chapters having such suggestive titles as "How Much Poison?", "Your Peck of Dirt," "Say It With Can-Openers," "Let The Housewife Beware," "Blood Money," "The Death Dealers," and "It's A Racket" vividly set forth various evils and abuses which have been and still are being encountered in connection with the distribution and advertising of foods and remedies. As with these, so with cosmetics ("Beauty At Cost"), the serious inadequacies of the present Food and Drugs Act are brought out, and the needs in this respect and the legislative situation to date of publication are discussed in considerable detail in the concluding chapter, "There's Going To Be a Law."

The fact that the latter prediction failed of fulfillment with the close of the last Congress is no reflection upon the accuracy of this prophecy. We should remember that the current act, now outmoded, came only after forty years of fighting, and in this case the battle for pure food and for honest labeling and advertising is to continue. Incidentally, if the injection here of this reviewer's opinion is pertinent, it may have been just as well that the recent measure failed of passage, embodying as it did the provision for divorcing from the Food and Drug Administration's control the vital feature respecting advertising—a proposal which inevitably must appeal to all food and drug officials as illogical, indefensible, and stupid.

"American Chamber of Horrors" is deserving of being read by every person interested in the advancement of this important cause of the protection of the American consumer.—C. D. HOWARD.

**The Romance of Chemistry**, by WILLIAM FOSTER, Professor of Chemistry at Princeton University, 497 pages, 31 full page illustrations, 32 figures in text. D. Appleton-Century Co. Price \$3.00.

This is the revised and enlarged second edition of a book that has enjoyed wide popularity for nearly a decade. Although written primarily for the layman and non-technical, it contains much of interest and value to the chemist who cannot be a specialist in every phase of his science. Material not always easy to locate elsewhere is presented here in very readable form, as is done, for example, in the chapter on "Cold Light." The author has put into language that will hold the attention of the uninitiated such important chemical subjects as "Electrons, Atoms, and Molecules," "Electricity in the Service of Chemistry," "The Magic and Mystery of Catalysis," "The Wonders of Radium," "Structure of Matter and the New Alchemy," "Bubbles, Drops, Grains, and Films: Colloid Chemistry." The last five chapters consider the Dependence on Chemistry of The Farmer, The Housewife, The Physician, The Manufacturer, and The Archaeologist, respectively. Each chapter is provided with "Reading References" to standard books and recent journal articles. The typography of the book is excellent and the illustrations (including several of historic interest) are all clearly reproduced. The book is cordially recommended to every student and teacher of chemistry.—LOUISE MCDANELL BROWNE.



**SECOND DAY**  
**TUESDAY—MORNING AND AFTERNOON**  
**SESSIONS—CONTINUED**

**REPORT ON DRUGS**

By L. E. WARREN (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

Twenty-one topics were assigned to associate referees in the drug section last year at the annual meeting, and one subject was assigned after the meeting. About the middle of the year the referee called the attention of the associate referees to the importance of completing as many topics as possible before the autumn meeting in order that the newly adopted methods might be published in the fourth edition of *Methods of Analysis*. Early in October the referee sent a second letter of the same import.

Progress was reported this year on eighteen subjects and reports submitted by the associate referees. No work was done on four. As a result of these studies, six methods were adopted as tentative. One other method gave satisfactory results and was recommended for tentative adoption by the associate referee but since it had already been accepted for the U.S.P. XI as a result of the associate referee's findings, it was not adopted by the Association. All subjects of the previous year that were not closed were continued.

A summary showing the status of the work at the close of the annual meeting is given herewith:

*Radioactivity of foods and drugs.*—The referee approves of the four recommendations made by the associate referee.

*Microchemical methods for alkaloids.*—Previous to this year the Association had adopted microchemical methods for the identification of the following 24 alkaloids:

|              |              |             |
|--------------|--------------|-------------|
| Aconitine    | Codeine      | Pilocarpine |
| Arecoline    | Ephedrine    | Procaine*   |
| Atropine     | Heroin*      | Quinidine   |
| Brucine      | Homatropine* | Quinine     |
| Caffeine     | Hyoscyamine  | Scopolamine |
| Cinchonidine | Morphine     | Sparteine   |
| Cinchonine   | Nicotine     | Strychnine  |
| Cocaine      | Papaverine   | Yohimbine   |

\* The synthetic alkaloids heroine, homatropine, and procaine were studied before the topic "Microchemical Methods for Synthetics" was undertaken by the Association.

In addition, lobeline and physostigmine have been studied, but no satisfactory microchemical tests for these alkaloids were developed and the topics were dropped.

This year the associate referee and his collaborators studied theobromine and theophylline and recommended that the subject be studied further with the view to developing confirmatory tests. However, the referee is of the opinion that the methods for theobromine and theophylline should be approved for adoption as tentative.

Since anabesine, apomorphine, hydrastine, and pelletierine are the only alkaloids having any extended medicinal or insecticidal use that have not been studied microchemically by the Association, it is recommended that a study of these four alkaloids be undertaken.

*Microchemical methods for synthetics.*—In addition to the recommendations of the associate referee, which are approved, the referee recommends that microchemical methods for barbital, phenobarbital, amytal, and ethylhydrocupreine (optochin) be studied.

*Hypophosphites.*—This topic has been studied for four years. Last year a method for total phosphates was adopted, but this did not differentiate between hypophosphites and phosphates. This year an attempt was made by the associate referee to determine hypophosphites in presence of phosphates by oxidation with standardized iodine. By varying the conditions of time and temperature a maximum of 92 per cent of theory was attained. The associate referee's recommendation that the topic be continued is approved with the suggestion that various other oxidizing agents be tried, such as bromine, perchloric acid, ammonium persulfate or perhydrol.

*Santonin.*—This subject (which includes santonica) has been studied for a number of years. A method for the assay of santonica was adopted in 1915, *This Journal*, 2, 52 (1916), and one for the determination of santonin in tablets and mixtures was adopted in 1930, *Ibid.*, 14, 51, 321 (1932). Still later, another alternative method for the determination of santonin in tablets was adopted, *Ibid.*, 18, 87 (1935). By the last-mentioned method the santonin is precipitated by dinitrophenylhydrazine sulfate and the compound is weighed.

This year the associate referee adapted the dinitrophenylhydrazine method to the assay of santonica. His report shows that the method used gives good results in collaborative tests. His recommendations are approved.

*Benzyl Compounds.*—This topic has been under consideration for four years. Most of the work has been done in attempting to devise methods for the assay of benzyl alcohol in mixtures, but to date no methods have been found satisfactory for adoption. No report was submitted by the associate referee, as no collaborative work was done.

*Rhubarb and Rhaponticum.*—The detection of rhaponticum in rhubarb has been under consideration for several years, but no methods have been adopted by the Association. No formal report was submitted by the associate referee this year. In a verbal report he indicated that progress had

been made in a crystallization test for detecting rhaponticum and recommended continuation of the topic. This is approved.

*Hexylresorcinol*.—This topic has been assigned for four years. A bromination method for the determination of hexylresorcinol was studied the first year, but the results obtained by collaborative work were not sufficiently consistent to warrant adoption. Since then the associate referee has been unable to do any work on the subject. It is recommended that the topic be reassigned.

*Ergot Alkaloids*.—This topic has been under consideration for four years, and considerable progress has been made. Last year a method for the colorimetric determination of the total ergot alkaloids was adopted, *This Journal*, 18, 88 (1935). The recent discovery of a new, very active substance in ergot suggested that attempts be made to develop a color reaction for its identification. Progress was reported in this direction, but the results were not sufficiently advanced to warrant collaborative work.

*Guaiacol*.—This topic has been under consideration by the Association for five years, but without substantial progress. Although the associate referee reports that it would be unwise at this time to continue the study, the referee is of the opinion that the work should be continued.

*Biological Testing*.—No work on pituitary gland was done this year, but the associate referee reports that he hopes during the coming year to carry out studies on the keeping qualities of the standard, powdered, posterior lobe of the pituitary gland. Some studies on the bioassay of aconite are in progress and the associate referee hopes to have a report for next year.

*Iodine Ointment*.—Iodine ointment has been studied for three years, *This Journal*, 17, 53, 464 (1934); 18, 53 (1935). The iodine in this ointment exists in at least three states, *viz.*: (1) the free; (2) inorganically combined (KI); and (3) organically combined. During the first year the associate referee developed methods for determining separately the free iodine and the potassium iodide, but no method was worked out for the organically combined iodine. In the second year a method was developed for determining the total iodine but only a little work was done in a collaborative way. Because of this fact the results were not reported until this year. They are in good agreement. A slight loss in total iodine noted after the year of storage could not be accounted for. No further work was done on the methods for free iodine or potassium iodide.

The associate referee recommends that the method for total iodine be adopted as tentative, but in view of the fact that the Committee of Revision voted to include the method in the U.S.P. XI (to become official June 1, 1936), it is questionable whether the method should be adopted. The referee believes that the studies should be continued with the view to developing methods for determining uncombined and combined iodine.

*Acetphenetidin in Presence of Caffeine and Aspirin*.—The associate



referee and his collaborators did considerable work on this difficult problem this year, but the task was not completed. The associate referee recommends that the method for the separation of acetylsalicylic acid from acetphenetidin be adopted as tentative and that the methods for the separation of acetphenetidin and caffeine be studied further. The referee believes, however, that it would be better to continue the subject until methods for the separation of the three constituents have been devised and studied collaboratively.

*Pyridium*.—The associate referee reports that some work was done this year but not sufficient to warrant a formal report. It is recommended that the subject be continued.

*Gums*.—The separation and identification of gums has been studied for three years, but no satisfactory method of identifying this material in mixtures has been found. The associate referee believes that progress has been made and that the work should be continued.

*Oil of Peppermint*.—To obtain more satisfactory results in the hands of different analysts, the U.S.P. assay methods for menthyl acetate and menthol were subjected to collaborative tests on oils of authentic origin and good quality. As a result of the study the associate referee recommended some slight but distinctly useful modifications to the U.S.P. assay procedure.

*Psyllium*.—The results of the collaborative trials this year were as satisfactory as could be expected. The recommendation of the associate referee that the method be adopted as tentative is approved, and it is further recommended that the subject be closed.

*Dinitrophenol*.—This topic was studied for the first time this year. A thorough review of the literature was made. The bromination process of assay was applied to dinitrophenol by the associate referee, but not to other preparations of the substance. It is recommended that the subject be continued.

*Theobromine Calcium*.—This is a new topic. Theobromine calcium is a preparation of theobromine in which the alkaloid is rendered partially soluble by lime. It is chiefly marketed under the name, theocalcin. The associate referee and his collaborators applied the periodide method to the assay of this product with excellent results. The recommendation that the method be adopted as tentative is approved, and it is also recommended that the topic be continued with the view to applying the method to tablets.

*Chlorbutanol*.—This is a new topic. The associate referee devised two methods for the assay of the substance. In one, the chlorine is broken from its organic combination by hydrolysis with alkali, and the chloride is then determined volumetrically by the Volhard process. The other is an application of the U.S.P. X method for the assay of acetone by oxidation with iodine. The results were promising, although but one collaborator

assisted the associate referee. In view of the small amount of collaborative work done, the recommendation of the associate referee that the subject be continued is approved.

*Aspirin and Phenolphthalein Mixtures.*—This is a new subject. The associate referee devised a method by which the acetylsalicylic acid (in aqueous suspension) is neutralized by sodium bicarbonate and the phenolphthalein extracted by chloroform. The phenolphthalein is weighed as such or determined as tetraiodophenolphthalein. The aqueous solution is then acidified and the acetylsalicylic acid extracted by chloroform. This is determined by the double titration or bromination process. This method appears promising, but no collaborative work was done. The subject should be continued.

*Stability of Potassium Iodate Volumetric Solution.*—This is a new topic, which arose when the stability of potassium iodate solutions on storage was questioned. Observations by the associate referee and his collaborators made quarterly on a standardized solution of potassium iodate over 9 months indicate that the solution is at least very nearly stable. The associate referee considers this observation time too short for final judgment, and his recommendation that the subject be continued is approved.

#### CHANGES IN METHODS

Several methods for the analysis of drugs having the status "Official, First Action" are described in *Methods of Analysis*. These have now been in use for over five years, mostly without criticism. Those that have not been criticized are determinations for the following drugs:

Acetylsalicylic Acid, Melting Point (XXXIX, 20).  
Ephedra (43).  
Camphor (55).  
Pyramidon Assay (98).  
Arsenic in Iron Methylarsenates (120).  
Mercurous Chloride in Tablets (128).  
Cat-eye Method for the Assay of Mydriatics and Myotics (150).

It is recommended that the methods listed above be made official, final action.

*Methods of Analysis* contains a number of methods that have the status of "tentative." These have been in the tentative status for over 5 years without substantial criticism. Those that have not been criticized are determinations for the following drugs:

Acetanilid and Caffeine (XXXIX, 5, 6, 7)  
Acetanilid, Caffeine, and Codeine (8, 9)  
Acetanilid, Caffeine, and Quinine (10, 11)  
Acetanilid, Caffeine, Quinine and Morphine (12, 13)  
Acetanilid and Sodium Salicylate (14, 15)  
Antipyrine and Caffeine (31, 32)  
Pilocarpine (37)

Emetine Hydrochloride in Tablets (38)

Atropine in Tablets (39)

Ephedrine in Inhalants (44)

Ephedrine in Tablets (46)

Apomorphine in Tablets (72)

Menthol (92, 93)

Thymol (94, 95, 96)

It is recommended that the methods of analysis for the drugs listed above be made official, first action.

*Arsenic in Iron-Arsenic Tablets—Tentative.*—This method has the status of "tentative" in *Methods of Analysis*, 1930, p. 477, par. 117, whereas the method for arsenic in iron methyl arsenate, which is official, uses a part of the tentative method (p. 477) to complete the assay. Since the iron methyl arsenate method is to become official, final action, it is recommended that the method for arsenic in iron-arsenic tablets be made official, final action, under suspension of the rules.

*Arsphenamine and Neoarsphenamine.*—Each of these drugs is described in the U. S. Pharmacopoeia, and an assay method for the determination of arsenic is given. This method is the same as that in *Methods of Analysis*, A.O.A.C., except for a very slight modification. It is apparent that the U. S. Pharmacopoeia has adopted the A.O.A.C. method essentially in full. To avoid duplication, therefore, it is recommended that the section entitled "Arsphenamine and Neoarsphenamine" in *Methods of Analysis*, 1930, pars. 121 and 122, be deleted.

*Barbital and Phenobarbital.*—An official method for the assay of barbital and phenobarbital is described in *Methods of Analysis*, 1930, p. 484, pars. 138 to 140, inclusive. It has been observed that if the official method be applied to tablets that contain small amounts of stearic acid in addition to the medicament, the results will be high. This is because the method fails to provide for the elimination of the stearic acid. It has also been found that if the residue of barbital (or phenobarbital) be purified by means of barium hydroxide solution the stearic acid may be eliminated.

It is recommended that the procedure suggested by the referee be adopted as an alternative tentative method for the assay of barbital and phenobarbital. This method has been published, *This Journal*, 19, 106 (1936).

In the assay of barbital and phenobarbital preparations the official method requires a special solvent composed of alcohol 20 volumes, ether 10 and chloroform 70. Barbital and phenobarbital are readily soluble in chloroform (1 in 110 and 1 in 60 parts, respectively). Inquiries among numerous chemists show that they regularly employ chloroform or a mixture of chloroform and ether in routine analyses instead of the A.O.A.C. special solvent. Therefore it is recommended that the special solvent be changed to ether, 20 volumes, and chloroform, 80 volumes.

*Chaulmoogra Oil*.—Methods for the detection of excess fatty acids and castor oil in chaulmoogra oil are described in *Methods of Analysis*, XXXIX, 142 to 145, inclusive.

In order to avoid duplication, since these methods will shortly be incorporated in the U.S.P. XI, it is recommended that they be deleted from *Methods of Analysis*.

*Dextrose in Ampuls*.—The referee is advised that a method for the assay of dextrose in ampuls is to be described in the new edition of the National Formulary. In this case the referee recommends the omission of this tentative method, *This Journal*, 15, 454 (1932), from *Methods of Analysis*.

*Ephedrine, Qualitative Tests For, by Copper Sulfate-Sodium Hydroxide—Tentative*.—The associate referee calls attention to the fact that the information and tests on ephedrine in *Methods of Analysis*, 1930, p. 456, par. 48, are to be incorporated in the U.S.P. XI substantially as given in the A.O.A.C. publication. His recommendation that paragraph 48 be deleted is approved.

*Mercuric Iodide Tablets—Tentative*.—The new National Formulary will describe a method for the assay of mercuric iodide tablets. This method is essentially the same as the A.O.A.C. method (XXXIX, 66, 67). In order to avoid duplication, the referee recommends that the A.O.A.C. method be deleted.

*Pepsin in Liquids*.—A tentative method for the assay of pepsin, specifying ricin as the reagent, was adopted by the Association in 1916, *Methods of Analysis*, 1916, p. 363. This method was not entirely satisfactory, and in 1917 it was voted that comparative work on the method should be resumed, *This Journal*, 3, 72, 527; 4, 19, 249 (1920). Apparently the matter was overlooked, for no further reports on the subject appeared. It now develops that only the highly purified product can be obtained in this country and that the price is very high.

Since the U. S. Pharmacopoeia describes a method for the assay of pepsin, it is recommended that the A.O.A.C. tentative method, *Methods of Analysis*, 1930, p. 464, be deleted.

Since the methods for the assay of pepsin, which use egg-white, have not proved entirely satisfactory, it is recommended that the edestin method be referred to the Referee on Enzymes for study.

*Phenolphthalein Tablets*.—An assay for phenolphthalein tablets, specifying iodine as a precipitant, has been official in *Methods of Analysis* for a long time, *This Journal*, 7, 14 (1923). This method will be described in the National Formulary VI. In order to avoid duplication it is recommended that the A.O.A.C. iodine method for phenolphthalein in plain tablets be deleted.

*Procaine Hydrochloride*.—In the official tests for the identification of procaine hydrochloride in *Methods of Analysis*, 1930, p. 471, par. 100,

there are three tests, viz. (d), (e) and (f), which are to be incorporated in the U.S.P. XI.

To avoid duplication it is recommended that these tests be deleted.

Two methods for the assay of procaine hydrochloride are described in *Methods of Analysis*, 1930, p. 471. In one the product is hydrolyzed by sodium hydroxide and the resultant product titrated with standard bromide-bromate solution; in the other the procaine base is extracted with a solvent and titrated with standard acid.

Procaine hydrochloride is now sold extensively in solutions in ampuls. The solutions may decompose with the formation of *p*-amino-benzoic acid. This product titrates with bromide-bromate solution like procaine.

(1) It is recommended that Method I in *Methods of Analysis*, 1930, p. 471, 101, be amended by inserting at the beginning the expression: "This method determines as procaine any *p*-amino-benzoic acid formed from the decomposition of procaine."

(2) It is recommended that Method II be amended by inserting at the beginning the expression: "This method determines only undecomposed procaine."

(3) It is further recommended that the title of the subject in *Methods of Analysis* be changed from "Procaine hydrochloride" (Novocaine) to "Procaine."

*Indicators in the Titration of Quinine.*—Methyl red is used as indicator in the titration of quinine (*Methods of Analysis*, pp. 442 and 473). Wales<sup>1</sup> has found that bromocresol purple is more desirable as an indicator for this purpose. It is recommended that the words "methyl red indicator" be changed to read "bromocresol purple" on each of the pages above mentioned. The directions for preparing bromocresol purple solution have been published, *This Journal*, 19, 105 (1936).

*Separation of Acetphenetidin and Caffeine.*—In the separation of acetphenetidin and caffeine (*Methods of Analysis*, 1930, p. 444, par. 16), the acetphenetidin is hydrolyzed to phenetidin sulfate under certain carefully controlled conditions. Many complaints have been received that the method gives low results for acetphenetidin, owing to incomplete hydrolysis. It has been found that if the evaporating and diluting process be repeated until acetic acid can no longer be detected by the odor or by moistened red litmus paper (i.e., until hydrolysis is complete) the results are satisfactory.

It is recommended that the sentence, "The diluting and heating process must be repeated until acetic acid can no longer be detected in the vapors," be inserted after the words, "liquid amounts to 8–10 cc.," in 17(a), line 5.

*Strychnine in Tablets and Liquids.*—Methods for the volumetric determination of strychnine in tablets and liquid preparations, *Methods of Analy-*

<sup>1</sup> *Ind. Eng. Chem.*, 18, 390 (1926).

*sis*, A.O.A.C., 1930, 472, par. 106, by solution of the isolated alkaloid in an excess of standardized acid, followed by titration of the excess acid with standardized alkali, were adopted as official, final action, in 1922, *This Journal*, 6, 269 (1923). Later these methods were changed to a direct titration procedure with standardized acid, *Ibid.*, 4, 572 (1920). It is now recommended that the official volumetric method be amended to permit a back titration process as an alternative method. The method, as amended, has been published, *Ibid.*, 19, 106 (1936).

#### NEW TOPICS

*Aminophylline*.—Aminophylline is a combination of theophylline and ethylenediamine. It is recommended that the assay of this substance be studied.

*Cinchophen-sodium Bicarbonate Mixtures*.—Tablets of cinchophen containing sodium bicarbonate are on the market. This substance interferes with the assay of the tablets by the National Formulary method.<sup>1</sup> It is recommended that a study of an assay method for cinchophen tablets containing sodium bicarbonate be undertaken.

*Homatropine in Tablets*.—Homatropine salts find extended use in ophthalmology. No method for the determination of homatropine salts in ophthalmic tablets has been adopted, either by this Association, the U.S.P., the N.F., or the joint Contact Committee of the A.D.M.A. and the A.Ph.M.A. It is recommended that a study of this subject be undertaken.

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### REPORT ON RADIOACTIVITY OF FOODS AND DRUGS

By C. H. BADGER (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

The use of the gamma ray electroscope for determining radioactivity by direct comparison of the commercial samples with standards of known radium content is rapid and accurate. However, the commercial apparatus now available is not capable of determining radioactivity in as small amounts as is the emanation or radon method. This method has been in constant use in the Food and Drug Administration for several years and is regarded as being almost indispensable. A description of it has been published, *This Journal*, 19, 101 (1936).

#### RECOMMENDATIONS<sup>2</sup>

It is recommended—

(1) That the gamma ray method suggested by the associate referee be adopted as tentative.

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<sup>1</sup> N. F., 6, 373 (1935).

<sup>2</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 53, 101 (1936).

(2) That the emanation or radon method, described in the report of the associate referee for 1930, *This Journal* 14, 85 (1931), be adopted as official, final action.

(3) That the title of the chapter be changed to "Radioactivity."

(4) That the emanation method<sup>1</sup> be modified to provide for the presence or absence of appreciable quantities of barium sulfate.

## REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS

By C. K. GLYCART (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

As recommended last year the study of theobromine and theophylline was begun. Caffeine, previously reported, *This Journal*, 13, 315 (1930), was included this year for comparative tests, since the compounds are related; caffeine is 1:3:7 trimethylxanthine, theobromine, 3:7 dimethylxanthine, and theophylline, 1:3 dimethylxanthine.

According to the literature, theophylline<sup>2</sup> forms a crystalline platinum-chloride, aurichloride and mercurichloride; the aurichloride is in lemon-yellow needles.<sup>3</sup> In the preliminary work, however, characteristic crystals were not obtained by the reagents under the conditions found in microchemical methods. The results were as follows: theophylline and chlorplatinic acid, no precipitate; gold chloride, amorphous precipitate; mercuric chloride, no precipitate.

Ammoniacal silver nitrate reagent forms a gelatinous mass with theophylline, even in dilute solutions, from which spheres of radiating needles appear within a minute. The reagent was adapted from the commercial method for the separation of theophylline<sup>3</sup> and theobromine from caffeine. When dissolved in ammonia, theobromine also forms a gelatinous precipitate with ammoniacal silver nitrate reagent but the precipitate is amorphous in the cold solution.

Kraut's reagent forms crystals immediately with theobromine 1:200. In more dilute solution, 1:1000, caffeine, theobromine and theophylline form crystals of similar appearance with Kraut's reagent.

Directions for the tests; control specimens consisting of theobromine, theophylline, and caffeine; and also samples labeled Nos. 1, 2, and 3 for identification, were sent to the collaborators. Sample No. 1 consisted of theobromine; No. 2 of a 1:200 solution of theophylline; and No. 3 of a 1:200 solution of caffeine. The materials for controls and tests were products of a reputable manufacturer, and were considered sufficiently pure

<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 433, 4(b) (2).

<sup>2</sup> Allen, *Commercial Organic Analysis*, 5th ed., p. 322 (1929).

<sup>3</sup> Henry, *Plant Alkaloids*, 2nd ed., p. 336.

for the work. The directions for making the tests have been published, *This Journal*, 19, 102 (1936).

### RESULTS AND COMMENTS

*Robert D. Stanley, U.S. Food and Drug Adm., Chicago, Ill.* No. 1, Theobromine; No. 2, Theophylline; No. 3, Caffeine.

The alkaloids were readily identified by the method. However, it was noticed that at greater dilutions than specified theophylline gave crystals with Kraut's reagent that were similar to those obtained with theobromine.

*Morris L. Yakowitz, U.S. Food and Drug Adm., San Francisco, Calif.* No. 1, Theobromine; No. 2, Theophylline; No. 3, Caffeine.

Each of the samples gave tests exactly as described.

*Samuel Alfend, U.S. Food and Drug Adm., St. Louis, Mo.*—No. 1, Theobromine; No. 2, Theophylline; No. 3, Caffeine.

*Irwin S. Shupe, U.S. Food and Drug Adm., St. Louis, Mo.*—No. 1, Theobromine; No. 2, Theophylline; No. 3, Caffeine.

The identification was based on comparison with controls as follows:

|   | HgCl <sub>2</sub>            | KRAUT'S                           | AMMONIACAL AgNO <sub>3</sub>   |
|---|------------------------------|-----------------------------------|--|
| Caffeine<br>(1-200 in H <sub>2</sub> O)     | long<br>radiating<br>needles | amorphous                         | —  |
| Theobromine<br>(1-200 in HCl (1+3))         | —                            | groups of<br>radiating<br>needles | Ag Cl  |
| Theophylline<br>(1-200 in H <sub>2</sub> O) | —                            | granules                          | Small dense groups of<br>radiating needles from ge-<br>latinous ppt. |
| No. 1 {                                     | 1-200 in HCl                 | groups of<br>needles              | Ag Cl  |
|   | Sat. H <sub>2</sub> O Soln   | groups of<br>needles              | —  |
| No. 2                                       | —                            | granules                          | Gelatinous ppt. small<br>dense groups of needles                     |
| No. 3                                       | long<br>needles              | amorphous                         | —  |

A water solution saturated with theobromine when tested with Kraut's reagent formed crystals of the same appearance as those formed in the presence of hydrochloric acid.

In the test for theophylline, crystals began to form in about  $\frac{1}{2}$  minute. It was not considered necessary to use a test tube. The identifications were readily made by comparison with controls. Descriptions of the crystals conform to what I saw in the tests.



## SUMMARY

The collaborators identified the alkaloids correctly. The test devised for the identification of theophylline by ammonium silver nitrate reagent is considered satisfactory. The immediate formation of crystals with Kraut's reagent and theobromine 1:200 appears sufficient to distinguish theobromine from its allies when compared in similar concentrations of 1:200.

It is recommended<sup>1</sup> that theobromine and theophylline be further studied with respect to confirmatory tests.

REPORT ON MICROCHEMICAL METHODS  
FOR SYNTHETICS

By IRWIN S. SHUPE (U. S. Food and Drug Administration,  
St. Louis, Mo.), *Associate Referee*

The three reports on the subject of Microchemical Methods for Synthetics made to this Association include identification tests for the following nine synthetics: benzocaine, chinosol, cinchophen, pyridium, anti-pyrine, amidopyrine, methenamine, triethanolamine, and dinitrophenol.

This year acetanilid, acetphenetidin, and neocinchophen were studied as a group since they are in that class of neutral compounds extracted by immiscible solvents from acid or alkaline aqueous solutions.

The devising of tests for acetphenetidin and neocinchophen was complicated by their relative insolubility in aqueous solutions. Hydrolyzing acetphenetidin and acetanilid to produce aniline and phenetidin sulfates solved the problem of solubility for these two, but the difficulty of hydrolyzing small quantities made the tests unsatisfactory. However, it was found that 10 per cent hydrochloric acid would dissolve sufficient amounts of all three synthetics to make them readily sensitive to several reagents. In the cold, 10 per cent hydrochloric acid will dissolve approximately 1 per cent of acetanilid, 0.2 per cent acetphenetidin, and 0.2 per cent of neocinchophen.

For acetanilid, bromine as liberated from bromide-bromate and photungstic acid were found to be the most satisfactory reagents. For acetphenetidin, in addition to Wagner's reagent for the solution, the nitric acid test<sup>2</sup> on the dry powder was considered to be characteristic. Neocinchophen formed characteristic crystals with potassium thiocyanate and platinic chloride.

The acetanilid, acetphenetidin, and neocinchophen used, purified by recrystallization, were considered satisfactory for these tests. The ace-

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 53 (1936).

<sup>2</sup> Mayrhofer, *Mikrochemie der Arzneimittel und Gifte*, II Teil (1928).

tanilid and acetphenetidin complied with U.S.P. requirements<sup>1</sup> for purity and the neocinchophen conformed to the standards proposed for it in N.N.R.<sup>2</sup>

Directions for the tests, control specimens of the synthetics, and unknown samples for identification were sent to collaborators. The unknown samples, identified as Numbers I, II, and III, contained acetanilid, neocinchophen, and acetphenetidin, respectively.

The method has been published, *This Journal*, 19, 103 (1936).

#### REPORTS AND COMMENTS OF COLLABORATORS

The collaborators, all of the U. S. Food and Drug Administration, identified the synthetics correctly. The names of the collaborators and their comments follow:

*Samuel Alfend, St. Louis Station.*—In the bromine test for acetanilid the long threads at the edge of the drop are characteristic.

*R. L. Vandaveer, Chicago Station.*—The phosphotungstic acid seemed to give more clearly defined crystals with acetanilid than did the bromide-bromate reagent. The nitric acid reagent is preferred to that of Wagner's in identifying acetphenetidin.

*Harold Cannon, Chicago Station.*—The tests seem entirely satisfactory. Crystals corresponding to descriptions given and to control specimens were obtained without difficulty.

*Morris L. Yakowitz, San Francisco Station.*—The methods appear satisfactory in each case. It was a very simple matter to match the unknown samples with the corresponding known.

*J. P. Aumer and E. C. Deal, New Orleans Station.*—We obtained positive tests for only one synthetic in each of the samples. Upon mixing the samples, we were able to identify neocinchophen direct upon the mixture, but not acetanilid and acetphenetidin.

*H. R. Bond, Chicago Station.*—Crystallizations were well defined. Identification of acetanilid with phosphotungstic acid reagent preferable to that with bromide-bromate reagent, which produced a network of needle crystals with a relatively small number of small prisms. Also, acetphenetidin produced rosettes or clusters of small needles with bromide-bromate.

Although the tests are designed for the pure synthetics it is of interest to note that two collaborators reported the test for neocinchophen applicable to certain mixtures.

#### RECOMMENDATIONS<sup>3</sup>

It is recommended—

(1) That the microchemical methods for the identification of acetphenetidin, acetanilid, and neocinchophen be adopted as tentative.

(2) That the more important barbituric acid derivatives and optochin be studied.

(3) That the compounds of the class phenylenediamine and tolylenediamine be considered as a subject for study.

<sup>1</sup> U.S.P. X, pp. 9 and 11.

<sup>2</sup> New and Non-official Remedies, 1934, p. 146.

<sup>3</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 53 (1936).

## REPORT ON HYPOPHOSPHITES

By HENRY R. BOND (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

The subject of hypophosphites was recommended for continuation for the purpose of devising a method for the determination of hypophosphites and phosphates in the presence of each other. Perusal of available literature disclosed but one method that appeared practicable.<sup>1</sup> The procedure involved in this method follows:

Place 5 cc. of a 1 per cent aqueous solution of the hypophosphite in a 500 cc. glass-stoppered Erlenmeyer flask, together with a 25 cc. of 0.1 *N* iodine solution and 5 cc. of hydrochloric acid (1+10). Warm the mixture to 70°–80°C. for 15 minutes, cool, and shake to dissolve the iodine vapor in the flask. Add 10 cc. of warm 10 per cent sodium borate solution, and after 10–15 minutes acidify the liquid with 2–3 cc. of glacial acetic acid and titrate with 0.1 *N* sodium thiosulfate solution. 1 cc. of 0.1 *N* iodine solution = 0.00165 gram of  $H_3PO_3$ .

This assay and the use of a 1 per cent aqueous solution of calcium hypophosphite produced a maximum recovery of about 80 per cent. Variations in the amounts of standard iodine solution added and in the time allowed for reaction increased the percentage of recovery as follows: 50 cc. of 0.1 *N* iodine solution and temperatures of 70°–80°C. for 30 minutes produced recoveries of 86 per cent and 87 per cent; 25 cc. of the iodine solution and temperatures of 70°–80°C. for 90 minutes gave recoveries of 91.6 per cent and 92 per cent. Other variations were also used, but the 92 per cent recovery represents the maximum obtained by the associate referee.

Because of the low results obtained with this procedure, no samples were sent out for collaborative work.

## DISCUSSION

Theoretically, hypophosphite could be determined in a mixture of hypophosphite and phosphate by use of the iodometric assay, the hypophosphite being oxidized to phosphate as follows:  $H_3PO_3 + 2I_2 + 2H_2O \rightarrow H_3PO_4 + 4HI$ . However, the above assay, with the variations noted, was found to be inadequate for hypophosphites in aqueous solution.

The associate referee believes that the iodometric method cannot be used for the assay of any of the recognized hypophosphite sirups, and that the difficulty of determining hypophosphites and phosphates in the presence of each other in a sirup mixture would be increased by the presence of the sugar.

It is recommended<sup>2</sup> that the subject of devising a method for determining hypophosphites and phosphates in the presence of each other in a sirup be continued.

<sup>1</sup> *J. pharm. chim.*, [8], 18, 5 (1933).

<sup>2</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 53 (1936).

## REPORT ON SANTONIN

By HARRY J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

This year the work of the associate referee was confined to a study of the determination of santonin in santonica. Last year a proposed method was submitted to collaborators, *This Journal*, 18, 529 (1935). Results were not satisfactory. It was felt, however, that the method was correct in principle, and this year a modification of the same method was sent to several collaborators together with a sample of ground santonica. The modified method has been published, *This Journal*, 19, 104 (1936).

The method as originally submitted called for extraction with benzene for one hour, a period of time which the associate referee had found sufficient for complete extraction of the santonin. Early reports from some collaborators showed, however, that with their apparatus extraction of the santonin was not complete in this time. Requests were then made to assay the sample by the modified method, extracting the santonica with benzene for three hours. Results of collaborators are shown in Table 1.

TABLE 1.—*Santonin in santonica*

| COLLABORATOR   | EXTRACTION PERIOD WITH BENZENE | PER CENT SANTONIN FOUND |      |      |      |           |
|--|--------------------------------|-------------------------|------|------|------|-----------|
|  |                                | HOURS                   | 1    | 2    | 3    | 4 AVERAGE |
| S. W. Bower<br>Direct Sales Co., Inc., Buffalo,<br>N.Y.        | 3                              | 3.67                    | 3.76 |      |      | 3.72      |
| H. M. Burlage<br>Univ. of North Carolina, Chapel<br>Hill, N.C. | 3                              | 3.03                    | 3.06 | 3.27 |      | 3.12      |
| D. C. Grove<br>Food & Drug Adm., Baltimore,<br>Md.             | 3                              | 3.52                    | 3.41 |      |      | 3.47      |
| W. F. Reindollar<br>State Dept. of Health, Baltimore,<br>Md.   | 3                              | 3.19                    | 3.10 | 3.22 |      | 3.17      |
| I. S. Shupe<br>Food & Drug Adm., St. Louis, Mo.                | 1                              | 3.33                    | 3.30 |      |      | 3.32      |
| G. Smith<br>Food & Drug Adm., New York<br>City                 | 1                              | 3.21                    | 3.22 |      |      | 3.22      |
| A. Stitt<br>United Drug Co., Boston, Mass.                     | 2-2½                           | 3.49                    | 3.49 |      |      | 3.49      |
| H. J. Fisher   | 1                              | 3.45                    | 3.31 | 3.41 | 3.35 | 3.38      |
| Average  |                                |                         |      |      |      | 3.36      |

Agreement between collaborators is believed to be as satisfactory as could be expected. The average deviation from the mean value of 3.42 per cent is  $-0.07$  per cent. The absolute santonin content of the sample is not, of course, known. However, experiments of the associate referee in which 0.0900 gram of specially purified santonin was mixed with 3 grams of mugwort (*Artemisia vulgaris*) as a control and the mixture assayed by the method gave an average recovery of 0.0856 gram, or 95.3 per cent.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the proposed method for the determination of santonin in *santonica* be adopted as tentative.

(2) That the present tentative method for santonin in *santonica* (*Methods of Analysis*, A.O.A.C., 1930, 472) be deleted. The obviously unsatisfactory character of a method with a large solubility correction was commented on in last year's report.

(3) That the study of methods for the determination of santonin be discontinued.

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No report on benzyl compounds was given by the associate referee.

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No written report on rhubarb and rhaponticum was given by the associate referee.

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No report on hexylresorcinol was given by the associate referee.

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#### REPORT ON ERGOT ALKALOIDS

By C. K. GLYCART (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

The isolation of a new alkaloid of ergot this year has confirmed the clinical discovery made in 1932 by Moir<sup>2</sup> that ergot contained a substance possessing a prompt powerful action, which is not ergotoxine and ergotamine, hitherto supposed to be all important, but which in reality play a subsidiary part in the clinical action of the drug.

Now that it is known that the characteristic traditional effect of extracts of ergot, when administered orally, is due principally to the new alkaloid, a revision of the tentative colorimetric method seems desirable.

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<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

<sup>2</sup> *Brit. Med. J.*, June 18, 1932, p. 1119.

It is believed that the chemical method can be modified to include a quantitative separation from the ergotoxine type of alkaloids. According to the recent literature the new alkaloid also responds to the Smith-Allport reagent<sup>1</sup> (paradimethylaminobenzaldehyde-ferric chloride-sulfuric acid). It occurs only in minute quantity in ergot.

In view of the new development in the chemistry of ergot alkaloids, further study on the preparation of an independent permanent color standard was not made as recommended last year, *This Journal*, 18, 54 (1935). The work was limited to preliminary determinations to separate the alkaloids in a sample of extract of ergot according to the directions given in detail by Dudley and Moir<sup>2</sup> for ergometrine. A commercial product purchased on the open market, consisting of Ergotrate<sup>3</sup> Lilly 1/320 grain tablets, was examined by extracting first with ether from an ammoniacal solution according to the Smith colorimetric method.<sup>4</sup> After having been acidified with sulfuric acid, then neutralized with sodium carbonate, the solution was extracted with chloroform. A blue color was produced by the Smith-Allport reagent. Results of the preliminary findings must await further investigation.

It is recommended that the work be continued with the view to devising a method for the separation and determination of the active alkaloids of ergot.

## REPORT ON GUAIACOL

By H. WALES (U. S. Food and Drug Administration,  
Washington, D.C.), *Associate Referee*

A number of methods described in the literature for the determination of phenols are also applicable to guaiacol.

Unfortunately guaiacol is usually found in drug products mixed with other phenols, as for example creosote, which is a mixture of guaiacol and creosol. At the present time there is no reliable method for the separation and determination of small amounts of closely related phenolic bodies. Until such a method is devised it is recommended<sup>5</sup> that no further study be made of this particular phenol.

No report on biological testing was given by the associate referee.

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<sup>1</sup> M. R. Thompson, *J. Am. Pharm. Assoc.*, 24, 9, 749 (1935); M. S. Kharasch and R. R. Legault, *J. Am. Chem. Soc.*, 57, 1141 (1935); W. A. Jacobs and L. C. Craig, *Science*, 82, 2114, 16 (1935).

<sup>2</sup> *Pharm. J.*, 80, 709 (1935).

<sup>3</sup> The maleate of a new active principle (base) isolated from ergot.

<sup>4</sup> Public Health Rpts., 45, 1466 (1930).

<sup>5</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

## REPORT ON IODINE OINTMENT

By WM. F. REINDOLLAR (State Health Department,  
Baltimore, Md.), *Associate Referee*

The method for the determination of total iodine described by the associate referee last year was subjected to collaborative investigation. Samples were sent to collaborators with the request that two sets of determinations be made, the second after an interval of six months. The findings of the collaborators, together with the results of a number of determinations made in this laboratory during the summer of 1934, but withheld until they could be accompanied by figures from other laboratories, follow:

*Maryland State Health Department Results—1934*

| COLLABORATOR        | TOTAL IODINE—PER CENT |      |      |      |
|---------------------|-----------------------|------|------|------|
| John C. Krantz, Jr. | 7.01                  | 7.19 |      |      |
| William G. Cesky    | 6.95                  | 7.07 |      |      |
| Associate Referee   | 7.02                  | 7.02 | 7.12 | 7.00 |

*Collaborative Results—1935*

| COLLABORATOR             | TOTAL IODINE—PER CENT |      |            |      |
|--------------------------|-----------------------|------|------------|------|
|                          | FIRST SET             |      | SECOND SET |      |
| S. W. Bower, Buffalo     | 6.99                  | 6.95 | 6.92       | 6.90 |
| H. J. Fisher, New Haven  | 6.99                  | 6.94 | 6.95       | 7.00 |
| W. D. Dembeck, Baltimore |                       |      | 6.85       | 6.82 |
| G. Smith, New York City  | 6.80                  | 6.81 | 6.78       | 6.78 |
| H. R. Bond, Chicago      | 6.97                  | 6.91 | 6.75       | 6.77 |
| Associate Referee        | 6.91                  | 6.94 | 6.91       | 6.79 |

Late in September a third set of determinations made by the associate referee yielded 6.83 per cent and 6.87 per cent.

Early in the year the method was examined by the Committee on Assays for Ointments of the United States Pharmacopoeia (U.S.P. XI Bull. Subcommittee, p. 338) and is being considered for inclusion in the forthcoming standard.

Although the more recent figures indicate that the ointment is deteriorating slowly, no collaborator had any difficulty obtaining duplicate results. In view of this fact it is recommended<sup>1</sup> that the method for the determination of total iodine in iodine ointment be adopted.

REPORT ON ACETPHENETIDIN IN PRESENCE  
OF CAFFEINE AND ASPIRIN

By SOLOMON M. BERMAN (U. S. Food and Drug Administration,  
Philadelphia, Pa.), *Associate Referee*

The previous associate referee directed his efforts toward effecting a proper separation of the several constituents in a mixture of aspirin,

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

acetphenetidin and caffeine, retaining for the determination of the individual substances such methods as have already been adopted by the Association. These methods, however, are still tentative, although the youngest one was adopted as far back as 1924, and the eldest was described in 1909. While these methods have been successful in certain cases, they have not been generally satisfactory.

The tentative method for aspirin in such mixtures, *This Journal*, 8, 265 (1925), consists of an acid hydrolysis followed by chloroform extraction of the salicylic acid and caffeine, a sodium carbonate wash being used to recover the salicylic acid. This separation is essentially faulty in that the aspirin is not recovered as such and any other salicylate would be reported as aspirin. The separation is otherwise unsatisfactory in that the acetphenetidin is not completely hydrolyzed in the reflux apparatus, but is later found contaminating the caffeine residue. This separation was improved by the former associate referee to the extent of placing the alkali wash at the beginning. This permits the acetphenetidin hydrolysis to be made without the need for a reflux apparatus. Warren, *Ibid.*, 17, 466 (1934), expressed the opinion that the hydrolysis of acetphenetidin is reversed by the presence of acetic acid, minimizing the statement of Harrison and Glycart, *Ibid*, 8, 28 (1924), that the presence of chloroform is the retarding factor. These two points were dealt with experimentally, and will be referred to later in this report.

The associate referee had occasion to examine a regulatory sample of the character being investigated, in 1933, at about the same time that recourse to the official method for separation of salicylates from other phenols had to be made on another sample. The use of a bicarbonate solution for separating carboxyl-containing compounds from practically neutral substances seemed even more sound than its approved application. The separation of aspirin from acetphenetidin and caffeine was therefore attempted, and was found to be quite satisfactory. When carried through promptly the separation did not involve any material hydrolysis of the acetylsalicylic acid. These observations were reported independently by Hitchens,<sup>1</sup> who credits Dott<sup>2</sup> with having found that aspirin could be dissolved in dilute sodium bicarbonate solution, the solution acidified, and a fairly quantitative recovery of essentially pure acetylsalicylic acid made with a mixture of ether and chloroform.

Hitchens described a method whereby the aspirin is extracted from the acidified solution with ethyl acetate, which is then removed under reduced pressure. Chloroform is used throughout in the proposed methods, but the principle of the aspirin separation is the same, namely the high acidity of acetylsalicylic compared to carbonic acid, together with the practically neutral character of a fresh bicarbonate solution. Sodium

<sup>1</sup> *J. Am. Pharm. Assoc.*, 23, 1085 (1934).

<sup>2</sup> *Pharm. J.*, 122, 302, 355 (1929); 124, 214 (1930).



bicarbonate begins to decompose in aqueous solution at 15°C., and a stock solution could be expected to contain considerable normal carbonate, and consequently have an increased pH.

The hydrolysis of acetphenetidin was studied with a view to shortening the process and establishing a definite time for a given weight of sample; 0.2 gram of acetphenetidin was chosen as a convenient maximum. No attempt was made to find out how far the acid mixture could be concentrated beyond the 5 cc. limit advised by the tentative method, *This Journal*, 2, 49 (1916), for acetphenetidin in presence of caffeine. It may be mentioned that a pink color developed in a short time when acetphenetidin was heated with sulfuric acid (1+4) under pressure at 100°C. Three methods of applying the acid hydrolysis were tried out: (1) the open digestion, whereby 10 cc. of 1+9 acid is repeatedly concentrated to 5 cc., any crystallized matter being brought into solution with chloroform; (2) reflux digestion, in which the acid mixture is heated in a boiling bath with a little chloroform present, an air or water condenser being used; (3) distillation over a flame, water being added at the 5 cc. concentration. The first two methods were also tried without chloroform. The observations made are summarized.

Method (1) with chloroform:

Very slow; requires continual replacement of water and chloroform.

(2) Incomplete after 2 hours.

(3) Complete after distilling about 50 cc.  
Elapsed time less than 30 min.

Without chloroform:

Slow; requires frequent replacement of water.

95 per cent complete in 30 min. but equilibrated.

Two determinations by (3) yielded 99.7 and 99.6 per cent recovery by the gravimetric procedure. No chloroform-soluble residue could be obtained from the hydrolysis mixture, indicating satisfactory hydrolysis. However, it appeared preferable to improve (2) for routine work, since the distillation method did not lend itself conveniently to the subsequent operation of transferring the acid solution to a separator, besides needing a water condenser and dropping funnel as accessories. Therefore, method (2) was modified to stop the reflux after 30 minutes, long enough to boil off most of the acetic acid, and the hydrolysis was completed by refluxing for another 30 minutes. Chloroform had been found to be an absolute retarding agent, hydrolysis in its presence being providential rather than consequential. With the modified method the hydrolysis was completed in 1 hour, the only attention needed being the intermediate boiling mentioned. The distillation method was retained as a volumetric method, the acid in the distillate being titrated with standard alkali.

Even with complete removal of aspirin and acetphenetidin it was not expected that the caffeine could be weighed with any degree of accuracy,

in view of the small amounts found in such mixtures. Volumetric and gravimetric methods were attempted, based on the precipitation of caffeine by iodine in acid solution, with discouraging results. No experiments were made with buffers. Instead, recourse was had to a micro-Kjeldahl procedure, the digestion being according to Pregl,<sup>1</sup> while the distillation was modified from the description of Pucher, Vickery and Leavenworth,<sup>2</sup> the titration being after Meeker and Wagner.<sup>3</sup> While the method is not specific for caffeine, it is superior to the gravimetric method in not reporting non-nitrogenous matter, and in reporting only about 35 per cent of any acetphenetidin as caffeine. Recovery of caffeine was 100 per cent by this method.

The methods submitted follow:

#### ASPIRIN

Aspirin is converted to the sodium salt to permit the removal of acetphenetidin and caffeine by a chloroform extraction. If this separation is carried out with promptness, and the aspirin extracted at once from the aqueous solution, no material hydrolysis occurs. Therefore the residue obtained can be treated according to the double titration method. On the other hand, if the aspirin is in aqueous solution for more than one hour, or has been subjected to other decomposing influences, such as heat or contact with alkali, it is preferable to determine total salicylates by the official bromine method.

#### REAGENTS

(a) *Sodium bicarbonate solution*.—Stir several grams of sodium bicarbonate, C.P. or U.S.P., into about 10 parts of water, and filter. Prepare just before use. The reagent should be as free as possible from normal carbonate.

(b) *Chloroform*.—U.S.P.

(c) *Methyl orange indicator solution*.

(d) *Phenolphthalein indicator solution*.

(e) *Alcohol*.—U.S.P., neutralized to phenolphthalein.

(f) *Sulfuric acid*.—1+9.

(g) *Sodium hydroxide*.—0.1 N.

(h) *Sulfuric or hydrochloric acid*.—0.1 N.

#### Double Titration Method

Transfer to a small separator a quantity of the powdered sample to represent not more than 0.2 gram of acetphenetidin. Add 5 cc. of the bicarbonate solution, tilting the separator to avoid loss of spray, and 5 cc. of water. Swirl to dissolve the aspirin. When effervescence has ceased, add 50 cc. of chloroform and shake out for 1 minute. Draw off the chloroform into a second funnel and wash with a mixture of 1 cc. of bicarbonate solution and 4 cc. of water. When clear, filter the chloroform through a cotton plug in the stem of the funnel. Make two additional chloroform extractions, using 50 cc. each time, washing and filtering the extracts as before. Reserve the filtered extracts for the determination of acetphenetidin and caffeine.

At once combine the aqueous extracts in the first separator and add 1 drop of methyl orange indicator. Neutralize with the 1+9 acid, and add up to 1 cc. in excess, tilting the separator to avoid loss of spray. Extract the aspirin immediately with chloroform, using 30, 20, 20, 10, 10, 10 cc. portions. Combine the clear ex-

<sup>1</sup> Quantitative Organic Microanalysis, 2nd ed., p. 110 (1930).

<sup>2</sup> Ind. Eng. Chem. Anal. Ed., 7, 152 (1935).

<sup>3</sup> Ibid., 5, 396 (1933).

tracts in a second funnel and wash with 3 cc. of water. When clear, filter the chloroform through a plug of cotton in the stem of the separator, using a small beaker as receiver. Extract the wash water with 5 cc. of chloroform, added to the main extract.

Evaporate most of the chloroform on the steam bath, finishing without heat. Dissolve the residue in 10 cc. of the neutral alcohol and add 2-3 drops of phenolphthalein indicator. Titrate at once with 0.1 *N* NaOH to the first pervasive pink. Note the amount used, add an equal volume plus about 5 cc. in excess, and heat on the steam bath for 15 minutes. Titrate the excess of alkali with 0.1 *N* acid. The total alkali used should be double that of the first titration, if the aspirin residue is pure. 1 cc. 0.1 *N* NaOH = 0.009 g. of aspirin.

#### *Bromine Method*

##### ADDITIONAL REAGENTS

- (a) *Sodium hydroxide solution*.—5 per cent.
- (b) *Hydrochloric acid*.—C.P. or U.S.P.
- (c) *Bromine solution*.—0.1 *N*. Dissolve 3 grams of KBrO<sub>3</sub> and 12 grams of KBr in water, and dilute to 1 liter. Standardize against 0.1 *N* thiosulfate solution.
- (d) *Potassium iodide solution*.—Dissolve 20 grams of KI in 100 cc. of water. Protect from light.
- (e) *Thiosulfate solution*.—0.1 *N*. Recently standardized.

##### PROCEDURE

If the bicarbonate layer—after removal of acetphenetidin and caffeine—contains only starch, talc, or mineral salts, in addition to aspirin or its decomposition products, transfer it to a small beaker, add 10 cc. of the 5 per cent NaOH solution and heat on the steam bath for 10 minutes to effect complete hydrolysis of the aspirin. If other substances may be present, capable of absorbing bromine, repeat all the operations of the double titration method up to the point where a dry residue is obtained, and then make the hydrolysis. The solution remaining from the double titration may also be used, after evaporating all the alcohol. In that case further hydrolysis is unnecessary.

Cool the hydrolysis mixture, transfer to a 250 or 500 cc. volumetric flask, and make to the mark with water. Transfer an aliquot, to represent about 0.05 gram of aspirin, to an iodine flask of suitable size. Slightly acidify to release most of the carbon dioxide, make slightly alkaline again, and add an excess of the 0.1 *N* bromine solution. Add 5 cc. of the HCl, and quickly stopper the flask to prevent loss of bromine vapor. If necessary, cool under the tap. Shake the flask at intervals during 30 minutes, and then let stand for 15 minutes.

Add 5 cc. of the KI solution and quickly re-stopper the flask. Mix thoroly, wash down the sides of the flask with water, and titrate the liberated iodine with 0.1 *N* thiosulfate solution. 1 cc. of 0.1 *N* Br = 0.003 g. of aspirin.

#### ACETPHENETIDIN

Before acetphenetidin can be determined it must be hydrolyzed with acid to the corresponding phenetidin salt, so that the caffeine present may be separated with chloroform. The acetphenetidin may then be reformed and weighed; or the acetic acid split off in hydrolysis may be distilled and titrated with standard alkali. The titration may be checked, if desired, by completing the gravimetric procedure on the same sample.

##### ADDITIONAL REAGENTS

- (a) *Sodium bicarbonate*.—C.P. or U.S.P.
- (b) *Acetic anhydride*.—C.P., colorless.

*Gravimetric Method Only*

Reduce the combined chloroform extracts containing the acetphenetidin and caffeine, by evaporation on the steam bath or by distillation over a small flame. Transfer to a 200 cc. Erlenmeyer flask with the aid of a little chloroform, and evaporate to dryness on the steam bath. Add 10 cc. of sulfuric acid (1+9) and insert an air condenser through a foil-lined stopper. Immerse the flask in boiling water or steam, but keep the condenser well cooled with a draft of air. Digest for 30 minutes, take the flask from the bath, and remove the condenser. Heat the flask over a small flame, so that the acid solution just boils for about 2 minutes, but do not concentrate the liquid to less than 5 cc. Add 5 cc. of water, re-insert the condenser, and replace the flask in boiling water or steam for 30 minutes as before.

Cool the liquid, transfer to a small separator so that the final volume does not exceed about 20 cc., and complete the assay by the A.O.A.C. method, *Methods of Analysis*, 1930, p. 444.

*Rapid Volumetric Method*

Reduce to a small volume the combined extracts containing the acetphenetidin and caffeine, by evaporation on the steam bath or by distillation over a small flame. Transfer to a side-arm distilling flask, of about 120 cc. capacity, with a little chloroform and evaporate the solvent on the steam bath. Add 10 cc. of sulfuric acid (1+9), and connect the flask to a straight, water-cooled condenser, the delivery end of which dips into a 100 cc. graduated cylinder. Insert into the mouth of the flask a rubber stopper carrying a dropping funnel of at least 60 cc. capacity, the stem projecting slightly beyond the side-arm of the distilling flask. Place at least 60 cc. of water in the funnel.

Heat the flask gently over a small flame until the distillate measures 4 cc. It is desirable to protect from the flame that portion of the flask which lies above the 5 cc. of liquid, using for the purpose an asbestos square having a circular perforation about 2" in diameter. Drop in water from the funnel at the same rate that distillation takes place, so that the volume of liquid in the flask remains constant between 5 and 6 cc. Under normal atmospheric pressure and with the gentlest boiling the distillation goes at the rate of 2 cc. per minute. Distil 60 cc. and then start testing the distillate, as it drops from the condenser, with a strip of blue litmus paper. If the color is at all changed, continue the distillation until a neutral reaction is obtained. Ordinarily, with 0.2 gram of acetphenetidin or less, the distillation of 60 cc. is sufficient.

Transfer the distillate to an Erlenmeyer flask, together with the rinsings of the graduate cylinder. Titrate the acetic acid with 0.1 *N* NaOH, using phenolphthalein as indicator. 1 cc. of 0.1 *N* NaOH = 0.01792 g. of acetphenetidin.

Sulfuric acid spray in the receiver may be tested for prior to titration by adding a drop of neutral 10 per cent barium chloride solution and swirling for 2 minutes.

**CAFFEINE**

Caffeine is determined indirectly, by means of its nitrogen content. The proportion of nitrogen in caffeine is so large that the error due to the presence of other nitrogenous material, not removed by the previous separations, is largely minimized. A simple micro-Kjeldahl procedure is used.

**ADDITIONAL REAGENTS**

- (a) *Sulfuric acid*.—C.P. or U.S.P. Concentrated.
- (b) *Catalyst in digestion*.—Copper sulfate and potassium sulfate mixture; or selenium metal or compound.
- (c) *Sodium hydroxide*.—Saturated solution.

(d) *Boric acid*.—Saturated solution, colored a definite pink with methyl red indicator solution.

(e) *Sulfuric or hydrochloric acid*.—0.02 *N*.

#### PROCEDURE

Remove the bulk of the chloroform from the combined extracts containing the caffeine by evaporation on the steam bath or by distillation over a small flame. Transfer the last few cc. of solution to a test tube of heat resistant glass 12"×1", using a little more chloroform to rinse the original vessel. Evaporate the rest of the solvent in the test-tube just to dryness, and add 1 cc. of sulfuric acid to which a proportionate quantity of catalyst has been added. Heat the contents of the test-tube over a small flame, using an asbestos shield to protect the test-tube above the liquid from overheating. After carbonization begins, continue heating so that the liquid just boils, preferably using some form of condenser to prevent too great loss of acid by evaporation. A test-tube about 4" long, narrow enough to slip into the larger tube, and with a flared mouth to support it, serves quite well for this purpose. Heat for 15 minutes after the digestion mixture has cleared. If selenium was used a reddish turbidity may develop, which may be ignored.

Cool, and wash down the condenser and test-tube walls with 20 cc. of water. Cool again, and while cooling add a slight excess of the strong alkali through a long stemmed funnel. Insert in the test-tube a double bored rubber stopper, carrying in one hole a simple spray trap, and in the other hole a glass tube narrowed to a coarse capillary at the end, which should dip to the bottom of the liquid. This tube connects with a wash bottle containing dilute sulfuric acid for absorbing any ammonia in the air drawn through the system. In another test-tube 12"×1", insert a double bored rubber stopper, carrying in one hole a short piece of glass tubing which connects to the vacuum pump, and in the other hole a glass tube dipping to the bottom of the test-tube, and connected to the spray trap by the short length of rubber tubing. To absorb the evolved ammonia, add 10 cc. of the boric acid solution to the receiving test-tube. Surround the receiver with ice-water and draw air through the system at the rate of 3 bubbles per second as noted in the absorbing solution. Now immerse the distilling test-tube in boiling water for 30 minutes, so that the vapor also heats the spray trap. The efficiency of this system depends largely on keeping the whole distilling tube and spray trap hot. The ammonia is carried over by the heated air, and practically no water distills.

Disconnect the receiver from the rubber tube on the spray trap, and then release the stopper in the receiving test-tube or disconnect the tube to the vacuum. Do not turn off the vacuum while the stopper is in place.

Wash the delivery tube with a minimum of water, inside and out, into the boric acid solution. Titrate the ammonia with 0.02 *N* acid to an end point represented by 10 cc. of the boric acid solution in a similar test tube, diluted with water to the same level as the titrated solution. Run a blank on the reagents and the system, deducting from the titration obtained in the determination. 1 cc. of 0.02 *N* acid = 0.0009708 g. of anhydrous caffeine.

#### COLLABORATIVE WORK

Two samples were prepared for collaborative work, one from aspirin, acetphenetidin, and caffeine citrate, which had been assayed, and another by subdividing a sample of commercial compressed tablets labeled to contain 3.5 grains acetylsalicylic acid, 2.5 grains acetphenetidin, and 0.5 grains of caffeine. This latter sample, designated as "A" was the only one sent to collaborators, since the proper mixing of the authentic sample

required considerable time and testing. In the letter sent with the proposed methods, it was requested that a single analysis be made by each procedure, two being given for aspirin, two for acetphenetidin, and one for caffeine.

The reports and comments of the collaborators follow:

TABLE 1.—*Analysis of aspirin, acetphenetidin and caffeine in mixture*  
(Results expressed in percentage)

| COLLABORATOR | ASPIRIN |         | ACETPHENETIDIN |            | CAFFEINE (ANHYDROUS) |
|--------------|---------|---------|----------------|------------|----------------------|
|              | ALKALI  | BROMINE | GRAVIMETRIC    | VOLUMETRIC |                      |
| H. O. Moraw  |         | 48.8*   |                |            |                      |
|              | 46.0    | 48.5    | 33.8           | 37.5†      | 6.0                  |
| R. Hyatt     | 46.06   | 45.66   | 32.68          | 44.26*     | 6.44                 |
|              | 45.61   | 46.02   | 31.66          | 31.80      | 6.41                 |
| E. Lewis     | 46.0    |         | 31.8           | 34.1       | 16.2 (8.3 by         |
|              | 46.9    | 42.8    | 32.5           | 48.7†      | 13.5 weight)         |
| I. S. Shupe  | 46.0    |         | 32.4           |            | 4.8‡ (7.4 by weight) |
| S. M. Berman | 46.0    |         | 33.0           |            | 5.93                 |

\* On residue from double titration.

† Distillate contained sulfate.

‡ Distillation incomplete.

#### COMMENTS OF COLLABORATORS

*H. O. Moraw.*—There was a considerable amount of sulfate in the distillate that was titrated for acetic acid. This method requires a suitable spray trap before it can be depended upon.

*R. Hyatt*—The sodium bicarbonate separation for aspirin appears satisfactory and the usual bromination or titration may be carried out. The usual procedure of weighing the reformed acetphenetidin appears satisfactory and relatively simple and freer from danger of accidents than the distillation method. In the first distillation a flask of 80 cc. capacity was used. In spite of small flame and presence of glass beads, some bumping was noted, and a large amount of sulfates was present in the distillate. In the second distillation a 125 cc. flask was used with pumice to prevent bumping, and sulfates were absent from the distillate. The distillation rate was considerably slower than 2 cc. per minute.

A test with litmus was made when 45 cc. had been distilled and the distillate was found to be neutral. However, the contents of the distillation flask had been boiling for about an hour. It is believed (1) that powdered pumice should be used in the distillation flask; (2) the rate should be slower than 2 cc. per minute; (3) a flask of at least 125 cc. capacity should be used; (4) steam distillation would probably be safer; (5) the extraction and weighing of acetphenetidin would be the more satisfactory. With reference to caffeine, while digestion and aspiration of ammonia is certainly the longest way around, the method appears to give consistent results with a small blank correction. A 1.5"×12" tube was used for digestion and a 1"×10" tube as receiver.

*E. Lewis.*—You will note that one of the acetphenetidin and both of the caffeine results, as determined by distillation, are high. This might be attributed to a mechanical spraying which, in the caffeine determination particularly, is aggravated by passing air through the system.

*I. S. Shupe.*—I was particularly interested in the method of hydrolysis of acetphenetidin by refluxing. I did not expect it to completely hydrolyze the acetphenetidin, but tests on the caffeine residue showed no acetphenetidin. The caffeine was weighed as an approximation, for sake of comparison with the final nitrogen determination. The low result for caffeine (by titration of ammonia) may have been due to faulty technic since I found it practically impossible to keep the trap hot as directed.

### DISCUSSION

The fact that the double titration method for aspirin yielded consistent results indicates that this separation need not give trouble, the bromination requiring special precautions in the presence of carbonate. No definite conclusions can be drawn at this time as to the value of the gravimetric method for acetphenetidin, since the collaborators did not clearly indicate in all cases whether the reflux residue or the distillation residue was used. Hyatt's low result by the gravimetric method was obtained on the distillation residue, and was to be expected if there were loss by spraying. If Lewis' low result was obtained in the same way, and omitted from the column also, the new average would be 32.9 per cent with a deviation of 2.7 per cent above, and 1.5 per cent below the average. The working of the caffeine method evidently requires more careful definition.

### RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That the proposed separation of aspirin from acetphenetidin and caffeine be adopted as tentative.
- (2) That the methods for acetphenetidin and caffeine be further studied.

No report on pyridium was given by the associate referee.

### REPORT ON GUMS IN DRUGS

By J. H. CANNON (U. S. Food and Drug Administration,  
Chicago, Ill.), *Associate Referee*

Work on the separation of gums from drug products was continued during the current year, but no collaborative samples were sent out since no method studied has proved to be satisfactory.

While there is extensive literature concerning the colloidal substances, the material that has been reviewed by the associate referee deals principally with the behavior observed in solutions, or mixtures with water.

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

The present problem, however, is to separate the gums from mixtures that may contain a variety of substances and be prepared in a variety of ways. As the properties of the gum solutions, or emulsoids, may depend to a large extent upon the dispersion medium,<sup>1</sup> or solvent, employed and also upon the manner of preparation of the solution, it is to be expected that one method of separation may not apply equally well to all preparations. This has been the experience of the associate referee this year in the study of various methods. As an illustration is cited the result of an experiment with a well-known commercial preparation containing agar. At the point in the method where a heavy precipitate was always obtained with control preparations of agar, no precipitation occurred, although by special treatment the presence of a considerable amount of agar was confirmed. Several experimental procedures have been tried under varying conditions of acidity, concentration, and time, and data have been accumulated, but the results, although encouraging, indicate that much remains to be accomplished.

It is recommended that the study of the separation and identification of gums be continued.

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## REPORT ON OIL OF PEPPERMINT

By E. K. NELSON (Bureau of Chemistry and Soils,  
Washington, D. C.), *Associate Referee*

The work on essential oils was confined to a study of the analysis of peppermint oil, prompted by the receipt of a letter from a firm of commercial chemists, who reported a marked disagreement in the results of analysis of the same sample of oil by several different analysts.

In order to discover causes for these variations in results, three samples<sup>2</sup> of peppermint oil were sent out for collaborative work. It was directed that the method of the U. S. Pharmacopoeia be followed, and it was suggested that water-cooled condensers with ground glass joints be used if possible.

Results were reported by five analysts, as shown in the table.

## COMMENTS OF COLLABORATORS

Analyst I.—Our chemists remove the acetic anhydride after acetylation in the following manner: As directed in the Pharmacopoeia, we wash the acetylated oil with two approximately 15 cc. washes of distilled water, then successive 15 cc. sodium carbonate test solutions, until the aqueous layer is slightly alkaline to phenolphthalein (usually three washings). The acetylated oil is again washed with two small portions of distilled water, separated, dried, and filtered. The total time required for this washing is less than 30 minutes. We never had reason to believe that oil washed in this manner is not entirely free from acetic anhydride and is

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<sup>1</sup> Kruyt and Klooster, *Colloids*, p. 167 (1921).

<sup>2</sup> Kindly furnished by A. M. Todd and Co., Kalamazoo, Mich.



neutral. There is, however, as you suggest, a possibility that discrepancies might occur in imperfect washing and also during hydrolysis, although it is our opinion that hydrolysis would have little if any appreciable effect on this type of determination.

From our experience we are of the opinion that errors are more liable to arise from improperly standardized solutions or from differences of opinions as to when the end point has been reached in titration. We use our solutions rapidly due to the extent of our assaying oil of peppermint, but as a matter of laboratory routine we check them daily. The greatest possibility of error, however, we believe arises in

TABLE 1.—*Results of analysis of three samples of peppermint oil by five different analysts*

| ANALYST | SAMPLE NO. | OPTICAL ROTATION | SPECIFIC GRAVITY | REFRACTIVE INDEX | ESTER           | TOTAL MENTHOL   |
|---------|------------|------------------|------------------|------------------|-----------------|-----------------|
|         |            | <i>degrees</i>   |                  |                  | <i>per cent</i> | <i>per cent</i> |
| I       | 2003       | −24.35           | 0.9027           | 1.4610           | 8.25            | 58.4            |
| II      | 2003       | −24.55           | 0.9032           | 1.4608           | 8.33            | 57.54           |
| III     | 2003       | −24.54           | 0.9034           | —                | 8.10            | 58.42           |
|         |            |                  |                  |                  |                 | 57.82           |
|         |            |                  |                  |                  |                 | 58.25           |
| IV      | 2003       | −25.94           | —                | 1.4592           | 7.03            | 57.95           |
| V       | 2003       | −24.3            | 0.9028           | 1.4618           | 8.03            | 58.62           |
| I       | 2006       | −24.25           | 0.9023           | 1.4620           | 8.00            | 54.9            |
| II      | 2006       | −24.25           | 0.9022           | 1.4611           | 8.02            | 54.23           |
| III     | 2006       | −24.47           | 0.9026           | —                | 7.67            | 55.29           |
|         |            |                  |                  |                  |                 | 54.85           |
|         |            |                  |                  |                  |                 | 54.87           |
| IV      | 2006       | —                | —                | 1.4615           | 7.07            | 55.01           |
| V       | 2006       | −24.3            | 0.9017           | 1.4620           | 7.75            | 55.66           |
| I       | 2008       | −25.3            | 0.9024           | 1.4617           | 9.0             | 58.25           |
| II      | 2008       | −25.38           | 0.9030           | 1.4610           | 8.6             | 57.64           |
| III     | 2008       | −25.35           | 0.9032           | —                | 8.6             | 57.1            |
|         |            |                  |                  |                  |                 | 56.8            |
|         |            |                  |                  |                  |                 | 56.77           |
| IV      | 2008       | −25.94           | —                | 1.4591           | 7.62            | 56.95           |
| V       | 2008       | −24.6            | 0.9053           | 1.4620           | 9.2             | 59.4            |

titrating. Some saponified samples are light in color, and the end point is easily detected, while others have a reddish cast, which makes it difficult to detect the end point when using phenolphthalein as an indicator. We consider the end point to have been reached when the liquid loses its last trace of red color, disregarding entirely the presence of some red color in the precipitate formed.

Two samples of each lot being examined are run simultaneously for comparison during titration and as a check for the total menthol.

Analyst V.—In accordance with the referee's suggestions, condensers fitted with ground-glass joints were used in acetylation and saponification. An added precaution was taken in making the weighings of the oil and menthyl acetate by using stoppered flasks.

In connection with the titrations it was observed that the addition of some water before titrating facilitates the end point.

In making the determination of the blank on the alcoholic KOH would it be desirable to add an unsaponifiable neutral oil so as to more nearly approach the conditions as in the assay of the sample? (The associate referee thinks not.)

The initial menthol results of the associate referee (Analyst II) were two per cent lower than those of Analyst I. This was found to be due to the presence of water in the anhydrous sodium acetate, which is quite hygroscopic. With this sodium acetate results for menthol were: 2003 = 56.37, 56.28 per cent; 2006 = 52.66, 52.30 per cent; 2008 = 56.23, 56.22 per cent. A sample of this sodium acetate, heated in a test tube, evolved a considerable amount of water. When the work was repeated with freshly fused sodium acetate, the results were: 2003 = 57.54 per cent; 2006 = 54.23 per cent; and 2008 = 57.64 per cent.

A quantity of anhydrous sodium acetate, exposed in a beaker for a week, absorbed a large amount of water. Hence it is absolutely necessary to preserve anhydrous sodium acetate in well sealed bottles and to test it occasionally by heating a small amount in a test tube. If the presence of water is noted, the material should be fused again.

Another precaution that must be taken in the analysis of peppermint oil is to keep the samples in the dark in a cool place with a minimum of head space. In time the ester value appears to increase, as noted in the following results on samples stored in the refrigerator with large head space (exposure to oxygen).

| SAMPLE | AS RECEIVED | NINE MONTHS OLD | SAMPLES RETURNED BY |
|--------|-------------|-----------------|---------------------|
|        | (ESTER)     | (ESTER)         | ANALYST IV          |
|        | per cent    | per cent        | per cent            |
| 2003   | 8.33        | 8.65            | 8.88                |
| 2006   | 8.02        | 8.61            | —                   |
| 2008   | 8.60        | 9.02            | 10.22               |

### CONCLUSIONS

The results of this work indicate that there can be considerable improvement in observing the details of the methods, especially for menthol.

The proper care of the sample previous to analysis is highly important; all reagents used must be of the best quality, and the sodium acetate used as a catalyst must be absolutely anhydrous.

In the event that the saponified oil or the saponified acetylated oil is highly colored, the ten drops of phenolphthalein directed by the Pharmacopoeia are insufficient. It is therefore recommended in such cases to use 30–40 drops of the indicator, which will give a plainer end point.

The Pharmacopoeial method has been revised to emphasize the points brought out by this work and to include some slight changes. See U.S.P. XI, p. 259.

### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That oil of peppermint be analyzed very soon after it is collected.

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

(2) That if this is impossible the samples be stored in full bottles in a refrigerator in the dark.

(3) That if the samples taken for assay become highly colored during the analysis 30–40 drops of phenolphthalein T. S. be used as indicator instead of 10 drops.

(4) That these improvements be brought to the attention of the Revision Committee of the U. S. Pharmacopoeia.

(5) That the subject be closed.

## REPORT ON PSYLLIUM

By HENRY M. BURLAGE (University of North Carolina,  
Chapel Hill, N. C.), *Associate Referee*

The swelling factor of psyllium is indicative of the mucilage content, is of importance in evaluating the drug, and it also serves as a criterion in the identification of the various species which might be sold on the market for medicinal purposes. Attention in 1934 was devoted to the study of proposed methods for the estimation of this swelling factor, and the collaborative study, *This Journal*, 18, 557, (1935), indicated that the method of Youngken<sup>1</sup> is superior to that of Clevenger.<sup>2</sup>

However, the results obtained by the Youngken method showed considerable variation due, in part no doubt, to non-uniformity of mucilage content in the seeds as well as to varying amounts of impurities. From the observations made it also appears that a fairly constant and reasonably low temperature should be maintained during the swelling period in order to obtain a greater uniformity in volume readings and to minimize the chances for fermentation, which in some cases was reported to have occurred at the high temperatures prevailing, as most of the determinations were made during the summer months.

Accordingly the method and apparatus of Youngken were modified. The modified method has been published, *This Journal*, 19, 104 (1936).

Seven commercial samples obtained from two sources and identical to those used in the study of 1934 (with the exception of sample No.5) were subjected to a collaborative study by the modified method. Some of these samples are designated as "official," since they represent species that are recognized by National Formulary VI,<sup>3</sup> and others are designated "non-official," because they are not so recognized. This classification is made because the final bases for the adoption of the proposed method should be determined in the main by the applicability of the method in identifying and evaluating the official species of the drug.

<sup>1</sup> *J. Am. Pharm. Assoc.*, 21, 1272 (1932).

<sup>2</sup> *Drug Markets*, 29, 297 (1931).

<sup>3</sup> National Formulary VI, pp. 295-6.

The samples were identified and labeled as follows: No. 1, *Plantago Psyllium*; Psyllium, Spanish; No. 2-A, *P. arenaria*; Psyllium, Triple Cleaned Genuine French; No. 2-B, *P. arenaria*; Psyllium, French; No. 3-A, *P. ovata*; Plantago Seed, ovata Blonde; No. 3-B, *P. ovata*; Plantago, ovata Blonde; No. 4, *P. lanceolata*; Psyllium, German; No. 5, *Lallemantia Royeleana*; Lallemantia Seed Masone.

The results are shown in the table.

TABLE 1.—Official and non-official samples

| COLLABORATOR | OFFICIAL SAMPLES |        |     |      |      | NON-OFFICIAL SAMPLES |      |
|--------------|------------------|--------|-----|------|------|----------------------|------|
|              | 1                | 2-A    | 2-B | 3-A  | 3-B  | 4                    | 5    |
|              | cc.              | cc.    | cc. | cc.  | cc.  | cc.                  | cc.  |
| A            | 14.2             | 10.0   | —   | 11.5 | 11.5 | 4.9                  | 37.0 |
| B            | 14.4             | 11.4   | 9.8 | 11.5 | 11.3 | 5.0                  | 40.3 |
| C            | 13.5             | 8.5    | 8.5 | 12.5 | 12.0 | 4.0                  | 43.0 |
| D            | 13.8             | 13.0   | 9.6 | 12.3 | 12.0 | 4.1                  | 46.5 |
| E            | 13.9             | 8.7    | —   | 10.7 | —    | 4.2                  | —    |
| Average      | 14.0 —           | 10.3 + | 9.3 | 11.7 | 11.7 | 4.4 +                | 41.7 |

The collaborators in this study were John C. Krantz, Jr., Maryland Dept. of Health, Baltimore; Harry J. Fisher, Connecticut Agricultural Experiment Station, New Haven; H. W. Youngken, Massachusetts College of Pharmacy, Boston; C. B. Jordan, School of Pharmacy, Purdue University, Lafayette, Ind., and the associate referee.

### CONCLUSIONS

A study of the literature reveals no satisfactory method for the determination of the mucilage content of vegetable substances; the methods of Clevenger and Youngken seem to have some possibilities since they are rapid and may be carried out with a minimum outlay for apparatus. A previous collaborative study showed that the method of Youngken gives higher results than Clevenger's for reasons previously stated, but it seemingly does not give concordant results because of temperature variations, accompanied by some fermentation.

A modified method was studied and was found to give much better agreement for species of the drug recognized in the National Formulary VI and will serve to evaluate and identify these species.

It is recommended<sup>1</sup> that the method proposed be accepted as a tentative method for the evaluation of and the determination of the swelling factor of psyllium.

No report on dinitrophenol was given by the associate referee.

<sup>1</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 54 (1936).

## REPORT ON THEOBROMINE CALCIUM

By E. O. EATON (U. S. Food and Drug Administration,  
San Francisco, Calif.), *Associate Referee*

This problem was solved by W. O. Emery and G. C. Spencer<sup>1</sup> for soluble theobromine salts. Their method was used on this difficultly soluble calcium salt and found to be satisfactory with a few modifications.

A sample of this product in powdered form was sent to several collaborators with a request that they try the following method:

In a 50 cc. Erlenmeyer flask place 0.2 gram of the uniformly powdered and well dried (110°C.) sample and 2 cc. of glacial acetic acid. Boil by gentle heat and add 10 cc. of boiling water, then transfer to a 100 cc. volumetric flask with the addition of 10 cc. more of the boiling water. (The solution should be clear or nearly so when cooled.) Add 50 cc. of 0.1 *N* iodine solution and 20 cc. of saturated salt containing 2 cc. of hydrochloric acid and make up to volume. Shake well and let stand overnight. Filter, discarding the first 10 cc. Take a 50 cc. aliquot and titrate with 0.1 *N* thiosulfate solution.

The number of cc. of iodine solution consumed  $\times 0.0045$  = grams of theobromine in one-half of sample taken. (Apparently this is not a very definite salt, but it should contain about 44% of theobromine.)

The results of the collaborators are as follows:

|                 | <i>per cent by weight</i> |
|-----------------|---------------------------|
| E. O. Eaton     | 43.2                      |
|                 | 42.8                      |
| M. L. Yakowitz  | 42.7                      |
|                 | 42.7                      |
| H. O. Moraw     | 44.7                      |
|                 | 45.1                      |
| L. A. Salinger  | 44.8                      |
|                 | 43.0                      |
| R. L. Vandaveer | 44.4                      |
|                 | 44.9                      |
| Average         | 44.0                      |

## COMMENTS BY COLLABORATORS

*Yakowitz.*—The method is an easy one and no difficulties were encountered.

*Moraw.*—On the basis of only two determinations no comment can be made on the principles of the method. Regarding the directions, since there is no apparent reason for dissolving the sample in an Erlenmeyer flask, why not indicate a small beaker, as it is easier to transfer with limited amounts of water?

*Salinger.*—Hydrochloric acid added as directed precipitates sodium chloride. This was filtered and made up to volume with saturated salt solution.

It is recommended<sup>2</sup> that the method be adopted as a tentative method.

<sup>1</sup> *J. Ind. Eng. Chem.*, 10, 605 (1918).

<sup>2</sup> For report of Subcommittee B and action of the Association, see *This Journal*, 19, 55 (1936).

## REPORT ON CHLORBUTANOL

By F. C. SINTON (U. S. Food and Drug Administration,  
New York, N. Y.), *Associate Referee*

Chlorbutanol is a component of various remedies and has been used rather frequently as a preservative of ampul solutions. It is not described in the U. S. Pharmacopoeia. It will be official in U.S.P. XI, but no assay will be provided.

Denige's method, as described by Fuller,<sup>1</sup> is essentially a saponification with alkali in alcoholic solution and determination of the chloride. It was decided to make a study of this method, also to make an attempt to apply the U.S.P. method for acetone.

For purposes of experimental work a quantity of hydrated chlorbutanol was purchased from a reputable manufacturer. It was found to meet the British Pharmacopoeia requirements for purity and had a melting point of 78°C. The conversion to anhydrous form was not without difficulty, but after a number of trials, by distilling and discarding the first portion and then desiccating the distillate for a week, the associate referee obtained a product that melted at 96°C. A portion of this material was turned over to A. M. Allison, and analysis was made by the following methods.

## METHOD I

## REAGENTS

- (a) *Alcoholic potash*.—0.5 N.
- (b) *Nitric acid*.—Concentrated.
- (c) *Silver nitrate solution*.—0.1 N.
- (d) *Potassium thiocyanate*.—0.1 N.

## DETERMINATION

Transfer to an Erlenmeyer flask a sample equivalent to about 0.2 gram of chlorbutanol. Add 25 cc. of the alcoholic potash solution and reflux on a steam bath for 30 minutes. Cool, and transfer to a 200 cc. volumetric flask, washing with water. Make solution acid with the nitric acid and then add 5 cc. in excess. Add 50 cc. of the silver nitrate solution and make up to mark with water. After shaking thoroughly, pour through a dry filter, rejecting the first 20 cc. Titrate a 100 cc. aliquot with the thiocyanate solution. 1 cc. of 0.1 N  $\text{AgNO}_3$  = 0.005915 gram of  $\text{CCl}_3\text{COH}$  ( $\text{CH}_3$ )<sub>2</sub>. Correct for chloride in blank, if necessary.

## METHOD II

## REAGENTS

- (a) *Sodium hydroxide solution*.—4.3 grams to 100 cc.
- (b) *Hydrochloric acid*.—10 %.
- (c) *Iodine solution*.—0.1 N.
- (d) *Thiosulfate solution*.—0.1 N.

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<sup>1</sup> Chemistry and Analysis of Drugs and Medicines, p. 148.

## DETERMINATION

Weigh out a sample equivalent to about 0.3 gram of chlorbutanol and transfer to a 100 cc. volumetric flask. Add water, and when solution is complete, make up to mark and shake thoroughly. To a glass-stoppered flask add in the order named, a 30 cc. aliquot, 25 cc. of the sodium hydroxide solution, then 50 cc. of the iodine solution with constant shaking, and allow to stand for 15 minutes. Make acid with 16 cc. of the hydrochloric acid and titrate the residual iodine at once with sodium thiosulfate solution, adding starch as indicator when the liquid is nearly decolorized. Conduct a blank test with the same quantities of the reagents and subtract the quantity of iodine solution consumed in the blank test from that used in the assay. Each cc. of the difference which represents the iodine consumed by the reaction corresponds to 0.00296 gram of  $\text{CCl}_3\text{COH}(\text{CH}_3)_2$ .

The results follow:

|               | Method I        | Method II       |
|---------------|-----------------|-----------------|
|               | <i>per cent</i> | <i>per cent</i> |
| A. M. Allison | 99.2            | 99.7            |
| New York      | 99.0            | 99.2            |
| F. C. Sinton  | 99.5            | 99.2            |
| New York      | 99.2            | 99.5            |
|               |                 | 99.9            |

Allison comments that Method I seems to be entirely satisfactory, and that when Method II was tried with some variation as to order and time of adding various solutions it was discovered that results were not trustworthy unless directions were followed exactly.

## SUMMARY

Method I operated without any difficulty. With Method II it was found by both collaborators that it is essential to add reagents exactly in order as directed, variation leading to results as low as 60 per cent of theoretical. Furthermore the solution should be titrated after standing as directed. If the directions are followed closely, satisfactory results can be obtained.

While Method I may be sufficient in most cases, in view of the fact that there are many other organic chlorine compounds, it would be desirable to have Method II as a supplementary method. If analysis is made by both methods and the results check, the evidence would strongly indicate chlorbutanol.

It is recommended that further collaborative study of the methods be made, especially as to their applicability to chlorbutanol in mixtures.

No report on aspirin and phenolphthalein mixtures was given by the associate referee.

REPORT ON THE STABILITY OF POTASSIUM  
IODATE VOLUMETRIC SOLUTIONS

By SOLOMON M. BERMAN (U. S. Food and Drug Administration,  
Philadelphia, Pa.), *Associate Referee*

The stability of potassium iodate solutions has been questioned by Sinton, *This Journal*, 15, 420 (1932), although Jamieson<sup>1</sup> definitely states that the volumetric solution is permanently stable, and no other exceptions to this statement have been noted in the literature.

Since most of the reactions with standard iodate are carried out in strongly acid solutions, it would seem preferable to standardize the iodate solution under similar conditions. While the thiosulfate titration is perhaps the most convenient, it involves a reaction in dilute acid medium that is quite different in character. The possibility exists that a change in the composition of the iodate solution might show up in one of the reactions and not in the other. Accordingly it was suggested by A. G. Murray<sup>2</sup> that as a primary standard, either purified iodine or Bureau of Standards arsenic trioxide be employed, as both lend themselves to the familiar reactions in strong hydrochloric acid solution. The latter was selected as more convenient. Collaborators were requested to make up solutions of *M*/40 strength from any available potassium iodate, preparing a sufficient quantity to enable standardizations to be made quarterly over a period of two years. This length of time was believed to be sufficient to reveal any possibilities of deterioration. A method of standardization was selected from the literature,<sup>3</sup> with slight modification, and sent to the collaborators. Factors obtained for the first three quarters of 1935 are given in Table 1.

The method and directions submitted to collaborators follow.

## STANDARDIZATION OF POTASSIUM IODATE SOLUTION

## REAGENTS

- (a) *Hydrochloric acid*.—U.S.P. Concentrated.
- (b) *Arsenic trioxide*.—Resublimed or B. S. standard sample.
- (c) *Chloroform*.—U.S.P.
- (d) *Potassium iodate solution*.—*M*/40. Prepare as follows: Dissolve 10.70 grams of C.P. potassium iodate in distilled water and dilute to the mark in a 2 liter volumetric flask. Preserve the well mixed solution in a glass-stoppered bottle.

## DETERMINATION

Weigh accurately about 0.1 gram of arsenic trioxide, by difference, into a 250 cc. glass-stoppered flask. Add 30 cc. of the hydrochloric acid and swirl until complete solution is effected. (This may take several minutes.) Wash down the sides of the flask with 20 cc. of distilled water, cool the contents to room temperature, and add about 5 cc. of chloroform. Titrate with the potassium iodate solution, at first

<sup>1</sup> A.C.S. Monographs. Chemical Catalogue Co.

<sup>2</sup> Private communication, January, 1935.

<sup>3</sup> Treadwell and Hall, 7th Ed. (1930).



rapidly, until the liberated iodine is largely gone, and then slowly, stoppering the flask after each addition of iodate, and shaking the contents vigorously. At the end point the chloroform loses the last trace of pink due to free iodine, and is colorless, while the aqueous solution retains a pale yellow color. If more than 25 cc. of the iodate solution is used in the titration, add 10–15 cc. of the hydrochloric acid to maintain the necessary acidity. 1 cc.  $M/40$   $KIO_3 = 0.004948$  gram of  $As_2O_3$ .

NOTE: Report solution factors as of date standardized, and in addition, if possible, (a) the manufacturer of the iodate reagent, (b) the kind of bottle used for storing the solution, and (c) whether the solution was protected from exposure to light.

TABLE 1.—Standardization of  $M/40$  potassium iodate solution by  $As_2O_3$ 

| COLLABORATOR     | FIRST QUARTER | SECOND QUARTER | THIRD QUARTER |
|------------------|---------------|----------------|---------------|
| W. F. Reindollar | 1.000         | 0.997          | 0.997         |
|                  | 0.999         | 1.001          | 0.992         |
| H. O. Moraw      | 1.0005        | 0.9992         | 1.003         |
|                  | 1.0005        | 1.0000         | 0.9988        |
| S. M. Berman     | 0.998         | 0.999          | 1.001         |
|                  | 1.000         | 1.002          | 1.002         |
|                  | 1.002         |                |               |

The collaborators have expressed their willingness to continue this work for another year. It is recommended that the topic be continued for that length of time.

## REPORT ON CEREALS

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Referee*

The American Association of Cereal Chemists during the past year revised their book entitled "Cereal Laboratory Methods." It contains valuable methods, which are not in *Methods of Analysis*, A.O.A.C., 1930. It does not seem desirable to include all these methods in the chapter on cereals, but some of them will be of considerable service to those interested in cereal chemistry. Accordingly, it is recommended<sup>1</sup> that the following methods be adopted tentatively and taken from "Cereal Laboratory Methods": Original Ash of Phosphated and Self-rising flour (Gustafson method), Acidity of Alcoholic Extract (Greek or Balland method), and Total Carbon Dioxide in Self-rising Flour.

There seems to be no good reason for the two tentative methods for the determination of unsaponifiable residue (pp. 170 and 181). Recent work

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 64 (1935).

reported in the *Analyst*, 58, 203 (1933) has shown that all the sterols are not very soluble in petroleum ether and since the modified Kerr-Sorber method has shown good results on collaborative work it is recommended that the F.A.C. method be dropped.

The quantitative tentative method (No.1) for chlorine, p. 173, requires a special extraction apparatus, introduces a large blank, and is seldom used. Accordingly it is recommended that it be dropped.

The two methods for the determination of fat, Method I (ammonia) and Method 2 (acid), p. 178, do not give concordant results.

The ammonia method specifies ammonium hydroxide in the presence of alcohol and water to liberate the combined fat, whereas the acid method uses hydrochloric acid in the presence of alcohol and water. The methods are similar in other respects. The official method for lipoids gives a higher value than that obtained from Method I (ammonia) for fat. Apparently some of the lipoids are hydrolyzed by this ammonia treatment. The results by the ammonia method are higher than those by acid method No. 2. This is to be expected since nearly all the lipoids are hydrolyzed by the acid treatment. There seems to be no need for a method giving these intermediate results. It is therefore recommended that the ammonia method No. 1 be dropped.

It is recommended that the following note be added to the procedure for chlorides in ash as sodium chloride, p. 181: "This sodium chloride value deducted from the total ash does not give salt-free ash."

It is recommended that the method for fat (acid hydrolysis), pp. 178 and 181 under "Baked Cereal Products" and "Alimentary Pastes," respectively, be adopted as official, final action.

It is recommended that the official method for the extraction and identification of added color, p. 181, be dropped. The method has been modified and much improved. It is therefore recommended that the improved method submitted by J. F. Jablonski be adopted as official (first action). The referee concurs in the recommendations of the associate referees.

As there is no method for examination of products used in connection with the brewing industry, it is recommended that the question of the desirability of adopting the methods for examination of malt, prepared corn and rice products, corn grits, corn meal, brewers' rice, refined grits and refined flakes be referred to the Referee on Alcoholic Beverages.

The calculation of egg solids by the tentative method, p. 182, can not be relied upon to give accurate results due to the change of lipoid  $P_2O_5$  in the noodle on storage and also because this constituent in the eggs themselves may undergo change before they go into the noodles. It is therefore recommended that this method be deleted. It is recommended that the work on ergot in flour and  $CO_2$  in self-rising flour be continued and the subject of rye in flour mixtures be discontinued for the present.

## REPORT ON ASH IN FLOUR, MACARONI PRODUCTS AND BAKED PRODUCTS; CHLORIDES IN FLOUR AND BAKED PRODUCTS

By L. H. BAILEY (Bureau of Chemistry and Soils,  
Washington, D. C.), *Associate Referee*

### ASH IN FLOUR, MACARONI PRODUCTS AND BAKED PRODUCTS

In 1930, F. M. Walters<sup>1</sup> gave an account of new aids in the ashing of flour, in which he used some of the earth metals occurring in the third and fourth groups of the periodic system as catalysts. He used the nitrates of lanthanum, cerium, thorium and yttrium. Upon incineration these nitrates decompose into their respective oxides, which are not only non-volatile and non-hygroscopic, but render the ash of cereals infusible at ordinary ashing temperatures. They also serve to separate the ash into small aggregates, which are distributed over the bottom of the ashing dish. Without the addition of any such aid, it is very difficult to burn the flour to a completely white color at the ashing temperature of 550°C. The ash usually remains as a dark compact mass, in the bottom of the dish, and while it may weigh the same as if it were completely white, the analyst always hesitates to consider the ashing completed as long as any dark color remains. Any agent that removes this doubt is an important aid. Walters found that by using sufficient of these nitrates to yield 5–20 mg. of the oxides of the respective alkaline earths he was able to ash 5 grams of flour at bright red and cherry red temperatures in from 11 to 23 minutes and obtain results that checked reasonably close with those obtained by the regular A.O.A.C. method.

In 1932, J. W. Bowen<sup>2</sup> described a method for quick ashing of flours, in which most of the carbon is burned off in a muffle furnace at a heat slightly above a dull red, the door of the muffle being kept open. When the flour has been burned to a dirty gray ash with no noticeable carbon remaining the dishes are removed and after cooling 10 drops of nitric acid (1+19) are added to moisten the ash. The dish is rotated and placed where the acid will evaporate but not boil. The dish is then replaced in the muffle, which has been allowed to cool to a faint dull red and the operation is completed at this temperature with the muffle door closed. Bowen does not state how much this procedure shortens the time of ashing, but claims the same degree of accuracy as for the slow burning method.

Other methods of ashing flours that make use of oxygen have been proposed: magnesium-acetate-alcohol, calcium acetate-oxygen, and calcium acetate alone, but all of them are attended with more or less difficulties and complicated procedure. No experiments have been conducted by the associate referee on these methods.

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<sup>1</sup> *Cereal Chem.*, 7, 83 (1930).

<sup>2</sup> *Ibid.*, 9, 158 (1932).

The method of Bowen was modified by the associate referee so as to ash the samples at 550°C., the temperature used in the official A.O.A.C. method, rather than at the indefinite temperature given by Bowen, but the time required for ashing was not shortened enough to compensate for the additional handling of the samples.

The use of the salts of the alkaline earths seemed to offer possibilities worth investigating. First an attempt was made to ash both soft and hard wheat flours at the regular ashing temperature of 550°C. with the addition of 10 cc. of the nitrate solutions of such dilution that each 10 cc. contained approximately 15 mg. of the respective oxide. The results obtained by this procedure were unsatisfactory. Experiments were then repeated by using a temperature of 850°C., which is quite a bright red. At this temperature the ashing seemed to be completed in from 30 to 45 minutes.

For this work platinum dishes about 65 mm. in diameter and 25 mm. in depth were used. Five grams of flour was weighed into the dishes, and 10 cc. of the nitrate solution was added with a pipet. Then the sample was stirred with a glass rod, the rod being cleaned with a small piece of ashless filter paper, which was then placed in the dish. The dish was placed on a steam bath and evaporated to dryness. It was then transferred to the muffle furnace, which was already at 850°C. The door of the furnace remained open until the flaming ceased, then the door was closed. When the ash appeared entirely white, *i.e.* in about 30 to 45 minutes, the dishes were cooled in a desiccator and weighed. The sample consisted of 5 grams of flour. The following results were obtained.

| AID USED               | HARD WHEAT ASH<br>per cent | SOFT WHEAT ASH<br>per cent |
|------------------------|----------------------------|----------------------------|
| Lanthanum nitrate      | 0.414<br>0.416             | 0.388<br>0.392             |
| Cerium nitrate         | 0.404<br>0.410             | 0.370<br>0.372             |
| Thorium nitrate        | 0.408<br>0.402             | 0.378<br>0.378             |
| Yttrium nitrate        | 0.414<br>0.414             | 0.386<br>0.380             |
| None (A.O.A.C. method) | 0.416                      | 0.388                      |

From the few results presented it appears that there is a tendency for slightly lower results with cerium and thorium salts than with lanthanum or yttrium, these latter salts yielding values identical with those obtained by the official ashing method. These slight differences might not, however, be discernible in a larger number of analyses.

The results obtained indicate that collaborative work should be done

with the use of these alkaline-earth nitrates as a means of shortening the time required to secure reproducible and dependable ash results.

It seems desirable that the Association should have an alternative rapid method for determining ash that would be "official," the same as it has for moisture and some other determinations; therefore it is recommended that the method of using thorium nitrate or other alkaline-earth salt as an accelerator be adopted tentatively as a quick ashing method. It is also recommended that further work be done with rapid methods of determining ash in flour, macaroni products, and baked products.

#### CHLORIDES IN FLOUR AND BAKED PRODUCTS

The determination of a salt-free ash is not so simple a matter as it appears. H. B. Near of the Pacini Laboratories<sup>1</sup> has outlined a method for determining salt-free ash in which he determines the alkalinity of the ash by titrating with 0.1 *N* sulfuric acid and calculating the results in terms of sodium carbonate, then determines the chlorine in the neutralized ash solution and calculates the result to sodium chloride. The sodium carbonate and sodium chloride are added together and the sum subtracted from the total ash to obtain the salt-free ash.

This method is based on the hypothesis that upon ignition proteins give off ammonia, which reacts with the sodium chloride present to form sodium hydroxide and ammonium chloride, the sodium hydroxide combining with carbon dioxide to form sodium carbonate, and that this sodium carbonate must be taken into account in obtaining a salt-free ash.

The associate referee has done some work with this method, but the results are not all that could be desired.

In the case of macaroni products, chemists sometimes determine the  $P_2O_5$  in the ash and then, by using a factor, calculate the ash of the original flour or semolina used in the product. Such a method manifestly would not be accurate for baked products that contain phosphorus-bearing constituents other than the flour.

It is recommended<sup>2</sup> that further study be given to the determination of salt-free ash.

#### REPORT ON H-ION CONCENTRATION

By GEORGE GARNATZ (The Kroger Food Foundation,  
Cincinnati, Ohio), *Associate Referee*

In taking over the referee work on H-ion concentration of flour, baked products and alimentary pastes, the work for 1934-5 was planned largely on the basis of the 1933-4 studies and recommendations, *This Journal*, 18, 563 (1935). It appeared from the report of the former associate referee that more collaborative work would be desirable in order to

<sup>1</sup> *National and American Miller*, Nov. 1934.

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 64 (1936).

gain a more discriminating insight into the various factors and conditions affecting the accuracy of the test.

Accordingly, it was decided to submit samples to a somewhat larger group of collaborators under conditions that would eliminate preconceived ideas concerning the pH of the various samples, utilizing color standards that were prepared by the associate referee, and following out the directions as given in the tentative method, including not only the latest recommendation of centrifuging the extract, but providing for measurements of pH on extracts *not* centrifuged but filtered after settling and prior to introduction into the H-ion vessels. The indicators used previously were retained in these tests; namely, paranitrophenol and gamma (2:5) dinitrophenol.

### EXPERIMENTAL

Samples of flour, bread, cake, and macaroni, together with coded color standards, the two indicators, three H-ion vessels, and detailed directions were sent to the following collaborators, who made their determinations within a space of three days.

- (1) H. M. Simmons, Mid-West Laboratories, Columbus, Ohio.
- (2) C. O. Oppen, Lawrenceburg Roller Mills, Lawrenceburg, Ind.
- (3) H. V. Moss, Provident Chemical Works, St. Louis, Mo.
- (4) R. M. McKinstrie, The Allen & Wheeler Co., Troy, Ohio.
- (5) James Kelley, Lyon & Greenleaf Co., Ligonier, Ind.
- (6) E. G. Bayfield, Ohio Agr. Exp. Station, Wooster, Ohio.
- (7) L. M. Thomas, Keever Starch Co., Columbus, Ohio.
- (8) George Garnatz.

The samples were prepared as described by the former associate referee. The results reported by the collaborators are presented in the following table:

| COLLABORATOR      | FLOUR |       | BREAD |       | CAKE  |       | MACARONI |       |
|-------------------|-------|-------|-------|-------|-------|-------|----------|-------|
|                   | I*    | II†   | I     | II    | I     | II    | I        | II    |
| (1)               | 5.4+  | 5.4   | 5.2+  | 5.2   | 7.00- | 6.9-  | 6.1      | 6.0   |
| (2)               |       | 5.6+  |       | 5.6   |       | 6.8+  |          | 6.2   |
| (3)               | 5.2   | 5.2   | 5.1   | 5.1   | 6.8   | 6.8   | 6.0      | 5.9   |
| (4)               | 5.4   | 5.3   | 5.0   | 5.1   | 6.8+  | 6.8+  | 6.1      | 6.0   |
| (5)               | 5.2   | 5.2   | 5.4   | 5.5   | 6.8   | 6.8   | 5.7      | 5.5   |
| (6)               | 5.0   | 5.0-  | 5.0   | 5.0-  | 6.6   | 6.8   | 6.2      | 6.1   |
| (7)               | 5.6   | 5.4   | 5.2   | 5.2   | 6.8+  | 6.9+  | 6.0      | 6.0   |
| (8)               | 5.5   | 5.5   | 5.0   | 5.1   | 6.8   | 6.8   | 6.1      | 6.1   |
| Electrometric     |       |       |       |       |       |       |          |       |
| Method‡           | 5.49  | 5.4   | 5.06  | 5.04  | 7.24  | 7.23  | 6.16     | 6.16  |
| Average           |       |       |       |       |       |       |          |       |
| (colorimetric)    | 5.33  | 5.33  | 5.13  | 5.23  | 6.80  | 6.83  | 6.03     | 5.98  |
| Range             | 0.6   | 0.6   | 0.4   | 0.6   | 0.40  | 0.10  | 0.50     | 0.70  |
| Maximum deviation |       |       |       |       |       |       |          |       |
| from Average      | +0.27 | +0.27 | +0.27 | +0.37 | +0.20 | +0.07 | -0.33    | -0.48 |

\* Extract centrifuged and filtered.

† Extract filtered after settling.

‡ Obtained electrometrically by associate referee, using glass electrode.

## COMMENTS OF COLLABORATORS

*H. M. Simmons.*—Samples run on method No. 1 were more or less cloudy, while samples on method No. 2 were fairly clear solutions. The pH readings were slightly higher, in other words not quite so acid, on method No. 1 than they were on method No. 2. This is probably due to the fact that the cloudiness seemed to intensify the color. Using either method, I found the readings were rather hard to make on these two indicators.

*H. V. Moss.*—If we were determining the pH of the samples you sent we would not choose the color standards submitted, since the variations between them are too small to make for a reasonable degree of accuracy. Solutions from method No. 1 filtered more rapidly, but were more turbid than those from method No. 2. Perfectly clear solutions were not obtained on any of the samples by either method, making it necessary to place tubes of the original solution back of the standards in order to match the colors. No. 54 hardened filter paper was used.

*R. M. McKinstrie.*—Considerable difficulty was encountered on account of the cloudy condition of the filtrate, especially in the flour.

*James Kelley.*—I experienced considerable difficulty in obtaining a colorless extract from the bread and cake samples and think that this slight coloration may have caused an error.

*E. G. Bayfield.*—The flour filtrates were very dense and the cake filtrates were medium dense, giving in these two cases readings of doubtful value. In all cases the centrifuged samples filtered the fastest and were less clear than those which settled for 10 minutes. From these results I believe that the settling out method is superior. I ran the tests twice, in the first case by comparing the filtrate plus indicator against a light. However this method was unsatisfactory so I reran them, using a small comparator block that held four tubes suitably arranged so that the tubes contained water, known color standard, filtrate alone, and filtrate plus indicator. This proved more satisfactory than the first method, but even using this method I do not believe that unless clear filtrates are used the method is very satisfactory.

*L. M. Thomas.*—Given clear solutions and some sort of comparator to eliminate lighting variations these indicators would give more satisfactory results. It was found extremely difficult to make close comparisons with cloudy solutions. Everything considered we think there are better indicators available for these pH ranges.

## DISCUSSION

A—Despite the fact that errors due to preparation of indicator solutions and color standards were precluded, the results of the collaborators do not show good agreement. This indicates that the method as given does not adequately stipulate the conditions under which the determination is to be run. For example, it is apparent that some of the collaborators did not use the Walpole technic in comparing colors, while others experienced difficulty due to light conditions.

B—Fundamentally the method appears to have possibilities in the direction of yielding satisfactory results, since the average values of the colorimetric determinations agree fairly well with the electrometric determinations made by the associate referee. Moreover a study of the tabulated data and the comments of the collaborators indicate that when adequate precautions are observed, dependable results can be obtained.

C—It is apparent that additional indicators to cover the ranges above pH 7.0 should be included. This is brought out by the results reported for cake and becomes significant if baked products such as soda crackers are to be considered. On the sample of cake sent out no significance can be attached to any of the results reported since it is obvious that the pH lay beyond the range of the indicators used.

D—It is the associate referee's opinion, which finds confirmation in the comment of at least three collaborators, that filtering after settling yields a clearer extract than does filtration after centrifuging. This is worthy of consideration, as even in this work it became evident that centrifuges are not universally available.

E—Criticism against the recommended indicators is again in evidence. These probably can be overcome by setting up conditions within the test minimizing their disadvantages and at the same time utilizing the advantages previously pointed out.

F—It is evident from the data that, as in all analytical procedures, a reasonable degree of experience with the method is necessary before dependable results can be expected.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the tentative method be subjected to further study since it appears to be fundamentally sound.

(2) That the portion of the tentative method for the colorimetric determination of H-ion concentration, beginning "At the end of this time pour the extract," be revised to read as follows: "Let the flask stand quietly for 10 minutes, then decant the supernatant liquid through a hardened dry folded filter paper, rejecting the first 5 cc. and catching the rest of the extract in the H-ion vessels. Immediately determine the hydrogen-ion concentration by comparison with suitable colorimetric standards."

(3) That conditions of light for properly making color comparisons be studied.

(4) That indicators covering the higher ranges, as for example, meta-nitrophenol, be included in future collaborative studies.

(5) That the tentative method be re-written more explicitly and in greater detail to include: preparation of indicator solutions, preparation of colorimetric standards, method for preparing extract, specification of light conditions for color comparisons, and technic for eliminating effects of color and turbidity of extracts.

(6) That after it has been re-written, the tentative method be subjected to comprehensive collaborative study.

The associate referee not only wishes to express thanks to the various

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 54 (1936).



collaborators for their hearty cooperation but acknowledges as well the work done by W. Reiman in carrying out these studies and in the preparation of this report.

## REPORT ON DIASTATIC VALUE OF FLOUR

By M. J. BLISH (Agricultural Experiment Station of  
Nebraska, Lincoln, Neb.), *Associate Referee*

The purpose of this report is to present what the associate referee considers to be conclusive evidence as to the possibilities and the merits of the method which has been advanced to the status of official (first action). Specifications of the method, and the maltose conversion table to be used in connection with it, have been published in *This Journal*, 17, 394 (1934); 16, 503 (1933).

During the past two years, the Pioneer Section of the American Association of Cereal Chemists has conducted monthly collaborative tests of the method under consideration.

From 15 to 25 chemists, most of whom are associated with flour mill laboratories situated in Kansas, Oklahoma, Texas, Colorado, and Missouri, ordinarily participate in these collaborative trials. T. R. West, Chairman of the Pioneer Section of the A.A.C.C., has kindly permitted results of these tests to be used in this report.

For present purposes it is considered sufficient to report the collaborative results, respectively, for June, July, and August, 1935. These data are comparatively recent, they represent the work of analysts who are reasonably familiar with the method, and they involve flours of low, medium, and moderately high diastatic values, respectively. The data are shown in Table 1.

A mere glance at Table 1 is sufficient to convince anyone who is familiar with the ordinary requirements of modern milling and bakeshop procedure that the method affords both precision and reliability to a degree that is amply sufficient for the measurement and control of flour diastatic activity under any conditions that are likely to arise in industrial practice.

Recently Geddes and Eva<sup>1</sup> have reported the results of a critical study of the method in comparison with other methods, and with statistical analysis of their data. They conclude that "The Blish and Sandstedt improved method is to be preferred to the modified Rumsey method employing either gravimetric or volumetric procedures for the determination of reduced copper from the standpoints of greater convenience, lower experimental error, and better differentiation between flours."

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<sup>1</sup> *Cereal Chem.*, 12, 402 (1935).

Putnam, Blish and Sandstedt,<sup>1</sup> after recent further study, have noted certain special refinements and precautions that may be observed in cases where the highest possible degree of accuracy is desired.

TABLE 1.—*Maltese values from three series of collaborative tests*  
(Mg. maltose per 10 grams of flour in 1 hour)

| COLLABORATOR | JUNE SERIES | JULY SERIES    | AUGUST SERIES |
|--------------|-------------|----------------|---------------|
| 1            | 252         | 213            | 131           |
| 2            | 246         | 196            |               |
| 3            | 248         | <del>214</del> |               |
| 4            | 249         | 201            | 145           |
| 5            | 257         | 204            |               |
| 6            | 241         | 203            | 144           |
| 7            | 244         | 200            | 140           |
| 8            | 230         |                | 140           |
| 9            | 237         | 198            | 152           |
| 10           | 234         |                | 142           |
| 11           | 234         | 199            | 138           |
| 12           | 235         | 198            |               |
| 13           | 246         | 201            |               |
| 14           | 234         |                |               |
| 15           | 243         | 207            | 150           |
| 16           | 244         |                |               |
| 17           | 226         | 204            |               |
| 18           | 238         | 220            |               |
| 19           | 254         |                | 144           |
| 20           | 254         | 206            | 125           |
| 21           | 228         | 208            | 141           |
| 22           | 242         | 212            | 140           |
| Average      | 242         | 205            | 143           |

The associate referee recommends<sup>2</sup> that the method be given the status of official (final action).

## REPORT ON STARCH

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Last year it was recommended that the modified Rask method and modified Mannich and Lenz polarimetric method for the estimation of starch be studied collaboratively.

<sup>1</sup> *Cereal Chem.*, 12, 494 (1936).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 65 (1936).

The details of the modified polarimetric method were not available to the associate referee at the time of the report last year. This method applies solely to wheat starch and wheat products since only the specific rotation of wheat starch is reported. Accordingly, it was thought advisable to postpone collaborative study until further work is reported on the general application of the polarimetric method.

Rask published a note on the determination of starch in *This Journal*, 18, 502 (1935). He proposes to use an apparatus consisting of a 25×200 mm. thick-walled Pyrex test tube fitted with a Monel metal plunger piston. The diameter of the sphere of the piston should be 0.2–0.3 mm. smaller than the bore of the test tube. The variations in the starch results by the Rask method have been attributed primarily to the incomplete dispersion of the starch in acid solution. Therefore, the Rask theory is that if all the starch passes through the 0.2 mm. space between the plunger and the wall of the tube it will be completely dispersed in the acid solution. However, in actual application the results obtained by the associate referee showed less starch than by the modified Rask method, and a greater variation.

The method for the determination of starch published in *The Analyst*, 59, 673 (1934) is based on hydrolyzing the starch with 0.7 per cent potassium hydroxide solution at 90°C., and then finally precipitating and weighing the starch as starch iodide. The experience of the associate referee with the application of this method to wheat flour and whole wheat flour was not encouraging. Filtrations were very slow, and the results obtained were low and inconsistent. Attention is also called to two methods based on similar principles published by Denny, *Contributions from Boyce Thompson Institute*, 6, 129 (1934), and Sullivan, *This Journal*, 18, 621 (1935). The Sullivan method extracts the starch with boiling calcium chloride solution, precipitates the starch with alcohol, redissolves the starch in hot water, precipitates again with iodine in the presence of ammonium sulfate, hydrolyzes the starch iodide with hydrochloric acid, and determines reducing sugar. These methods have been worked out primarily for application to plant material, but apparently could be used on other products. However, the Sullivan method is long and involves many operations.

The authorities have not uniformly defined "starch." It therefore seems logical that different results are obtained by the many various methods for its determination. In view of this fact, and because the modified Rask method has received generally favorable comment with few exceptions and has given good collaborative results for two years, it is recommended<sup>1</sup> that the modified Rask method for the estimation of starch in cereals be kept as a tentative method and that the application to cereal products of the Mannich and Lenz and the Sullivan methods be studied.

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 64 (1936).

## REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration,  
New York City), *Associate Referee*

A study was made of three methods (those of Nicholls,<sup>1</sup> the Associate Referee,<sup>2</sup> and Munsey),<sup>3</sup> for the detection of benzoic acid in flour, for the purpose of increasing the amount of benzoic acid recovered, of obtaining consistent quantitative recoveries, and of getting a blank on unbleached flour with as little interfering color present as possible.

These results were accomplished by modifying the Munsey method in several respects. A better recovery was obtained by adding calcium chloride in the distillation procedure as used in the Nicholls method. It was found that amyl alcohol removes color from the distillate when alkaline. This color is difficult to oxidize and may cause interference in the blank. Superoxol (30% hydrogen peroxide) was used for purifying the distillate in order that the results of oxidation could be seen. If the distillate is not oxidized until practically colorless, poor blanks will be obtained, and if benzoic acid is present, the reddish color will be marked. Potassium permanganate, as used in the Nicholls and Munsey methods, is not always satisfactory.

The method as modified appears to give consistent quantitative results. Though collaborators were not asked for quantitative determinations, each one reported that Sample A contained twice as much benzoic acid as Sample B, which was correct. Tubis obtained correct duplicate recoveries on each sample and Ard and Hogan obtained the same recoveries as did the associate referee.

In unbleached wheat flours, the blanks gave no indication of the reddish color of *m*-diamine benzoic acid obtained in the modified Mohler test. Five authentic unbleached rye flours, however, had a faint pink hue, which would give an indication of 0.1 mg. of benzoic acid. The minimum recovery of benzoic acid in flour bleached with Novadelox would be over 1 mg., which gives a pronounced reddish color. If a blank of 0.2 mg. of benzoic acid be subtracted from the quantitative results obtained in all rye flours, no erroneous conclusions will be drawn.

Sample A contained approximately 35 mg. of benzoyl peroxide per kilo of flour; Sample B, one-half as much; and Sample C, none.

The results of five collaborators are correct and are as follows:

|   | SAMPLE A | SAMPLE B | SAMPLE C |
|---|----------|----------|----------|
| J. S. Ard, F. & D. Administration, New York               | Positive | Positive | Negative |
| L. H. Bailey, Bureau of Chemistry and Soils<br>Washington | Positive | Positive | Negative |
| J. L. Hogan, F. & D. Administration, New York             | Positive | Positive | Negative |

<sup>1</sup> *Analyst*, 58, 4 (1933).

<sup>2</sup> *This Journal*, 17, 302 (1934).

<sup>3</sup> *Ibid.*, 18, 489 (1935).

V. E. Munsey, F. & D. Administration, Wash-  
ington

Positive Positive Negative

Manuel Tubis, F & D. Administration, Phila-  
delphia

Positive Positive Negative

In *This Journal*, 18, 498 (1935), Munsey published satisfactory methods for the determination of chlorine in flour. It is recommended<sup>1</sup> that these methods be submitted for collaborative study during the coming year, and that the modified method for the determination of benzoic acid be adopted as tentative.

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A paper entitled, "Foreign Methods for Testing Brewing Materials," was presented by H. W. Rohde.

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No report on CO<sub>2</sub> in self-rising flour was given by the associate referee.

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The paper, "A Study of the Per Capita Bread Consumption," given by Washington Platt, Syracuse, N. Y., was published in *Northwestern Miller*, Jan. 1, 1936.

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No report on unsaponifiable constituents in macaroni products, bread and baked products was given by the associate referee.

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## REPORT ON FAT IN MACARONI PRODUCTS, BREAD AND BAKED PRODUCTS

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Mitchell's report on this subject, *This Journal*, 16, 301 (1933), indicates that the action of acid on the fatty substances of egg may be incomplete when hydrolyzed at 70°C. He reports that it was found necessary to raise the temperature to that of boiling water to insure complete splitting of the phosphoric-acid-choline group from the lecithin. It therefore seems desirable to compare the fat results obtained by the official acid hydrolysis method (temp. 70–80°) and at 100°C.

The following results were obtained on starch, flour, milk bread, and egg noodles:

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 64 (1936).

|         | OFFICIAL<br>ACID HY-<br>DROLYSIS<br>METHOD AT<br>75°C. | DRIED RESIDUE<br>FROM A, DIS-<br>SOLVED IN<br>PETROLEUM<br>ETHER | OFFICIAL<br>ACID HY-<br>DROLYSIS<br>AT 100°C. | DRIED RESIDUE<br>FROM C, DIS-<br>SOLVED IN<br>PETROLEUM<br>ETHER | UNIFIED<br>METHOD<br>WITH<br>WAX AT<br>100°C. |
|---------|--|--|---|--|---|
|         | A  | B  | C   | D  | E   |
| Argo    | 0.62   | 0.53   | 1.53  | 0.42   | 0.42  |
| Starch  | 0.58   | 0.41   | 1.89  | 0.43   | 0.47  |
|         | Av. 0.60   | Av. 0.47   | Av. 1.71                                      | Av. 0.43   | Av. 0.45                                      |
| Flour   | 1.30   | 1.26   | 2.12  | 1.23   | 0.75  |
|         | 1.33   | 0.98   | 1.96  | 1.19   | 0.89  |
|         | Av. 1.31   | Av. 1.12   | Av. 2.04                                      | Av. 1.21   | Av. 0.82                                      |
| Milk    | 5.10   | 5.08   | 5.70  | 5.20   | 4.79  |
| Bread   | 5.19   | 5.16   | 5.47  | 5.32   | 4.91  |
|         | Av. 5.15   | Av. 5.12   | Av. 5.59                                      | Av. 5.26   | Av. 4.85                                      |
| Egg     | 4.71   | 4.50   | 5.09  | 4.45   | 4.05  |
| Noodles | 4.71   | 4.62   | 5.32  | 4.41   | 3.99  |
|         | Av. 4.71   | Av. 4.54   | Av. 5.21                                      | Av. 4.43   | Av. 4.02                                      |

The results from hydrolysis at 100°C. are clearly too high, showing the presence of much non-fat material. This residue when dissolved in petroleum ether gave a much lower value. These results are expressed in Columns C and D. The results on hydrolysis at 75° are given in columns A and B. The material likewise weighed as fat gives a lesser value when the residue is dissolved in petroleum ether. This raises the question whether this extra step involved in dissolving the residue in petroleum ether is desirable when hydrolysis is at 70–80°.

In order to get some idea of the range of experimental error eight additional samples of the same egg noodles as reported in the previous table were analyzed for fat at 75°. The results obtained are as follows:

| A         | B   |
|-----------|---|
| per cent  | RESIDUE FROM COLUMN A, DISSOLVED IN PETROLEUM ETHER<br>per cent |
| 4.77      | 4.73  |
| 4.78      | 4.70  |
| 4.69      | 4.48  |
| 4.77      | 4.58  |
| 4.68      | 4.52  |
| 4.78      | 4.50  |
| 4.73      | 4.57  |
| 4.74      | (4.29)*   |
| 4.71      | 4.50  |
| 4.71      | 4.62  |
| Av. 4.74  | Av. 4.58  |
| Min. 4.68 | Min. 4.48   |
| Max. 4.78 | Max. 4.73   |

\* Not included in average.

The values in Column A, officially reported as fat, indicate duplicates will agree within 0.1 per cent of each other in products of this type, whereas the results obtained by dissolving the residue from Column A in petroleum ether vary 0.25 per cent. The reasons for this variation may be due to incomplete extraction of fat from the dried residue or some loss of fat involved in the several extra operations. The sample not included in the average evidently lost considerable fat. On this basis the difference in results from hydrolysis at 75° and 100° is within the limits of the method when the residue is dissolved in petroleum ether. Hydrolyzation was tried on the same samples with a temperature of 49–50°, but liquefaction of the material was so slight and so much of the material was still in lumps that the complete extraction of the fat would not be possible. For this reason extraction was not carried out.

A unified method for the determination of fat has been published by Fred Hillig, *This Journal*, 18, 454 (1935). This method is an acid hydrolysis method, different from the official method in that the fat material is absorbed by filter cel in the cold, and the filter cel is dried and then extracted with petroleum ether. In this case the fatty material is extracted in the presence of a solid phase, whereas in the official method it is extracted in the liquid phase in the presence of a mixture of petroleum ether, ether, alcohol, and water. The results on the same samples by this method are given in Column E. The flour, bread, and noodles give lower results, while the value for starch is practically the same. There are some objections to the unified procedure since it is necessary in many cases to add a known amount of wax in order that the fat be held by the filter cel. The procedure is much longer, requires more operations, and is more time-consuming. Recoveries of known amounts of fat are better than 90 per cent when the fat is added directly to the filter cel. The official method recovered 100 per cent of the fat added to purified starch.

#### DISCUSSION

The acid hydrolysis method has been studied collaboratively since 1926 with various reports since 1923. Palmer, *This Journal*, 8, 610 (1925), reports the maximum yield of fat for dried whole eggs at the temperature of 75–80°. As previously stated, Mitchell reports that for the determination of fat in eggs a temperature of 100° is necessary to split off all the phosphoric acid-choline group from lecithin. He shows, however, that at the temperature of 100° ether-extracted starch gives over 2 per cent fat which is actually non-fat material. Likewise, 71–74° temperature gives 0.15–0.1 per cent; fat hydrolyzation at 51–52° gives 0.04 per cent fat. Thus, while the temperature of 100° is high enough for estimation of the fat in eggs, too much non-fat material is extracted from products containing farinaceous material. A temperature of 70–80° does not hydrolyze off all the phosphoric acid-choline group from the lecithin, and it extracts

a slight amount of non-fat material. The temperature of 48–50°, at which practically no non-fat material is extracted, is too low to yield all the fat.

The last collaborative results by the acid hydrolysis method on egg noodles, macaroni, and bread were fairly satisfactory and showed improvement over the former results. The results reported in this paper do not warrant a change in the present method. Moreover, it seems very unlikely that the acid hydrolysis method can be modified to meet all the required conditions to determine the specific substance fat, namely, true glycerides. In view of this fact and the large amount of work already done on this subject it would seem that the present official acid hydrolysis method should be used to determine the substance that should be known as "fat by acid hydrolysis."

It is therefore recommended<sup>1</sup> that the study on the determination of fat in cereal products be discontinued for the present.

## REPORT ON MILK SOLIDS IN BREAD

By V. E. MUNSEY (U. S. Food and Drug Administration,  
Washington, D. C.), *Associate Referee*

Last year it was recommended that the modified citric acid method and the estimation of fat and "fat number" for the estimation of milk solids be further studied collaboratively.

The following method, based on the determination of citric acid and modified by Hartmann for the estimation of milk solids, was used in this year's collaborative work. The method used for the estimation of milk solids based on the determination of total fat and the percentage of butter fat present is only slightly changed from last year.

Three samples of bread were submitted to six collaborators with the request that moisture, fat, and "fat number," be determined by the fat method, and milk solids by the citric acid method. Bread No. 1 was a water bread with no milk solids; No. 2, a milk bread with 7.7 per cent milk solids; and No. 3, a half-milk bread with 3.9 per cent milk solids. All determinations were on a moisture-free basis.

The following results were obtained:

SAMPLE I

| COLLABORATOR | MOISTURE        | FAT<br>M.F.B.   | FAT<br>NUMBER | MILK SOLIDS<br>CALC'D FROM<br>FAT | MILK SOLIDS<br>CALC'D FROM<br>CITRIC ACID |
|--------------|-----------------|-----------------|---------------|-----------------------------------|---|
|              | <i>per cent</i> | <i>per cent</i> |               | <i>per cent</i>                   | <i>per cent</i>                           |
| 1            | 8.58            | 3.96            | 1.74          |                                   |   |
|              | 8.73            | 3.90            | 1.78          |                                   |   |
|              | Av. 8.65        | Av. 3.93        | Av. 1.76      | 0.31                              | 0.70                                      |

<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 64 (1936).



## SAMPLE I—Continued

| COLLABORATOR | MOISTURE        | FAT<br>M.F.B.   | FAT<br>NUMBER | MILK SOLIDS<br>CALO'D FROM<br>FAT | MILK SOLIDS<br>CALO'D FROM<br>CITRIC ACID |
|--------------|-----------------|-----------------|---------------|-----------------------------------|---|
|              | <i>per cent</i> | <i>per cent</i> |               | <i>per cent</i>                   | <i>per cent</i>                           |
| 2            | 8.82            |                 |               |                                   | 0.38                                      |
|              | 8.85            |                 |               |                                   | 0.47                                      |
|              | Av. 8.84        | 3.89            | 2.18          | 0.49                              | Av. 0.43                                  |
| 3            | 7.68            | 3.12            | 1.89          | 0.30                              | 0.11                                      |
| 4            | 8.60            | 3.88            | 2.85          |                                   |   |
|              | 8.64            | 3.83            | 2.80          |                                   | 0.35                                      |
|              | Av. 8.62        | Av. 3.86        | Av. 2.82      | 0.75                              | 0.35                                      |
| 5            | 8.63            |                 |               |                                   | 0.37                                      |
|              | 8.55            |                 |               |                                   | 0.32                                      |
|              | Av. 8.59        | 3.96            | 2.51          | 0.64                              | Av. 0.35                                  |

## SAMPLE II

|   |          |          |           |      |          |
|---|----------|----------|-----------|------|----------|
| 1 | 7.62     | 5.71     | 13.4      |      |          |
|   | 7.60     | 5.75     | 13.2      |      |          |
|   | Av. 7.61 | Av. 5.73 | Av. 13.3  | 7.5  | 8.2      |
| 2 | 8.55     |          | 11.98     |      | 7.57     |
|   | 8.52     |          | 12.35     |      | 7.53     |
|   | Av. 8.54 | 5.73     | Av. 12.17 | 6.82 | Av. 7.55 |
| 3 | 7.39     | 4.88     | 13.42     | 6.45 | 7.43     |
| 4 | 7.78     | 5.69     | 11.99     |      | 8.12     |
|   | 7.81     | 5.76     | 12.03     |      | 7.74     |
|   | Av. 7.80 | Av. 5.73 | Av. 12.01 | 6.72 | 7.84     |
|   |          |          |           |      | Av. 7.9  |
| 5 | 7.84     |          | 13.13     |      | 7.79     |
|   | 7.83     |          | 13.73     |      | 7.74     |
|   | Av. 7.84 | Av. 5.78 | Av. 13.43 | 7.64 | Av. 7.77 |

## SAMPLE III

|   |          |          |          |      |          |
|---|----------|----------|----------|------|----------|
| 1 | 7.16     | 4.99     | 8.12     |      |          |
|   | 7.47     | 4.93     | 8.38     |      |          |
|   | Av. 7.32 | Av. 4.96 | Av. 8.25 | 3.83 | 4.5      |
| 2 | 7.57     | 4.96     | 7.19     |      | 3.95     |
|   | 7.60     |          | 7.73     |      | 4.08     |
|   | Av. 7.59 |          | Av. 7.46 | 3.41 | Av. 4.02 |
| 3 | 6.90     | 4.60     |          |      | 3.24     |
| 4 | 7.60     | 4.96     | 8.24     |      |          |
|   | 7.68     | 4.94     | 8.12     |      |          |
|   | Av. 7.64 | Av. 4.95 | Av. 8.18 | 3.78 | 4.43     |
| 5 | 7.46     |          | 8.64     |      | 4.11     |
|   | 7.46     |          | 8.88     |      | 4.02     |
|   | Av. 7.46 | Av. 4.93 | Av. 8.76 | 4.07 | Av. 4.07 |

The results for determination of milk solids by both procedures are encouraging. The calculated amount of milk solids from the citric acid method agrees more closely with the amount present than do the results calculated from the fat method. With one exception the agreement in the total fat is excellent. The fat numbers are not in close agreement. In this study of the effect of the acid hydrolysis on fats Hillig found that wax must be added to the fat in order to get the same Reichert-Meissl number before and after acid hydrolysis. The associate referee found that the addition of wax before acid hydrolysis does give higher and more consistent fat numbers on the fat from some breads. It is noted that nearly all the milk solids calculated by the fat method are low.

The fat number on this water bread was higher than the average value found, but calculated milk solids less than one per cent have no meaning from the practical standpoint. It is believed that these methods are satisfactory for a tentative status, considering that average values were used for calculations on a product of the nature of bread, and that a slight modification will improve the fat method.

Thanks are due the following collaborators: J. H. Bornmann, F. and D. Administration, Chicago; F. B. Jones, F. and D. Administration, New York City; R. N. Gladding, Department of Agriculture and Immigration, Richmond; and D. C. Walden, Connecticut Agricultural Experiment Station, New Haven.

It is therefore recommended<sup>1</sup> that the citric acid and the fat method for estimation of milk solids in bread be adopted as tentative and that further studies be made. These methods have been published in *This Journal*, 18, 574 (1935); 19, 86 (1936).

No report on rye in flour was given by the associate referee.

## REPORT ON VISCOSITY OF FLOUR STANDARDIZATION OF MACMICHAEL VISCOSIMETER

By C. G. HARREL (Pillsbury Flour Mills Co.,  
Minneapolis, Minn.), *Associate Referee*

The standardization of a collaborative viscosity procedure by use of the MacMichael viscosimeter requires a standardization of the method of preparing the flour-water suspension, time of digestion, etc., and also a standardization of the MacMichael viscosimeter itself.

The report of the associate referee<sup>1</sup> in 1934 indicated that much of the variation of the viscosity results was due to the viscosimeter itself. From this work the following conclusions were drawn:

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<sup>1</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 65 (1936).

1. In making up the flour-water suspension, a mortar and pestle should be used. 2. An initial volume of 50 cc. of water should be used, and the total volume of water should be 100 cc. Less initial water makes for rapidity—and possibly smoothness—but causes low results. 3. The time of mixing the suspension should be  $2\frac{1}{2}$  minutes. 4. The suspension should be allowed to digest for 1 hour. 5. Any variation of results between different laboratories, using the same method of procedure, is most likely due to the viscosimeter itself.

It is believed that if viscosity is to be used as an index of the quality of gluten, the determination should be run on a definite protein basis. In the work done by the associate referee 2 grams of protein was used as a basis, that is the size of the sample is weighed out so that 2 grams of protein is present.

Concentrating on the MacMichael viscosimeter as a means of measuring viscosity of acidulated flour-water suspensions, the associate referee conducted the following work discussed in this report.

#### EXPERIMENTAL WORK

The first work was carried out to determine the effect of the distance between the bottom surface of the disc and the bottom of the bowl. In this report this distance will be referred to as "disc clearance." The effect of bowl speed was also noted. For this work, a 60 per cent sugar solution and three different flours were used. The method used in taking the viscosity follows:

Take such a weight of the flour that 2 grams of protein is present. Into a mortar place 50 cc. of distilled water, and on top of this put the weighed sample of flour. Mix the flour and water for  $2\frac{1}{2}$  minutes with the pestle, and place the suspension in a 250 cc. Erlenmeyer flask. Rinse the mortar and pestle with two 25 cc. portions of distilled  $H_2O$  and add the rinsings to the suspension in the flask. (This brings the total amount of water used up to 100 cc.) Allow the flour-water suspension to digest for 1 hour, shaking the suspension once at the half-hour mark. Add 9 cc. of 1 *N* lactic acid to the contents of the flask and shake. Allow the acidulated suspension to stand for 5 minutes, pour it into the bowl of the viscosimeter, and take the viscosity reading as soon as possible by dampening the swing of the dial.

The disc clearance was adjusted to the following distances: 0.05, 0.1, 0.3, and 0.5 inch. Bowl speeds were as indicated in Figs. 1, 2, and 3, which show the effect of bowl speed and disc clearance clearly.

The curves shown are common to plastic substances. It is noted, however, in Figs. 1, 2, and 3, that the curvature is in the lower bowl speed region of the graph and that the slope of the curve is also less in this region than at the higher bowl speeds. It is also noted that at the higher bowl speeds the graph is more or less a straight line. These curves indicate that better results would be expected at the higher bowl speeds than at the lower bowl speeds. That is, a variation of 1 r.p.m. at 12 r.p.m. would show a greater difference of viscosity than at 30 r.p.m.

In the graphs 4, 5, and 6, viscosity *vs.* disc clearance, it is noted that as the disc clearance becomes less, the curvature of the graph becomes

greater. For example, at any definite bowl speed, a variation of 0.05 inch in disc clearance at 0.1 inch produces a much greater variation in viscosity than a 0.05 inch variation at 0.4 or 0.5 inch disc clearance. By comparing results on the above data, it may also be noted that by making

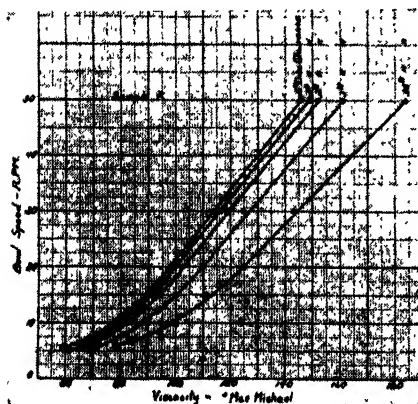


FIG. 1

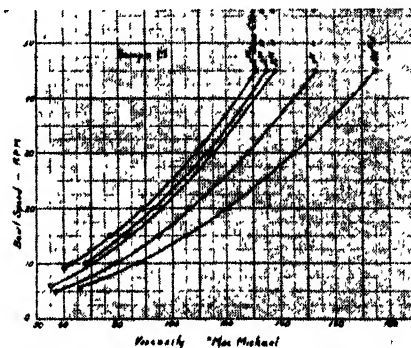


FIG. 2

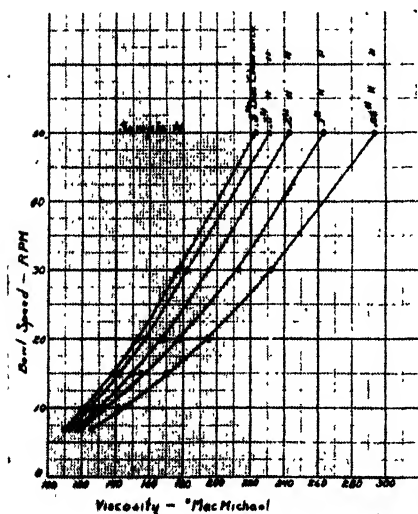


FIG. 3

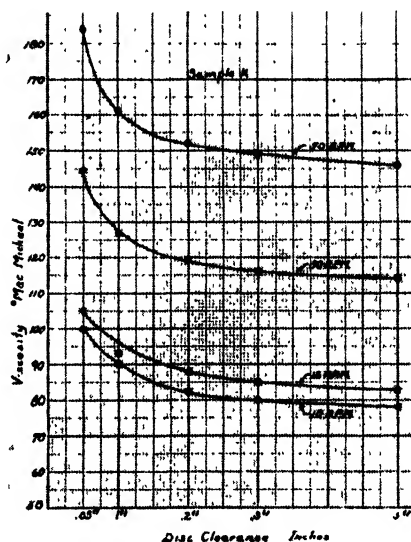


FIG. 4

the disc clearance 0.5 inch, the spread of viscosity between two different flours is about the same as at 0.1 inch disc clearance. Therefore, by moving the disc clearance out to 0.5 inch the sensitivity of the machine is not affected.

A 60 per cent sugar solution (by weight, sp. gr. 1.281 at 30°) was run

for viscosity at 30°C., and both the disc clearance and bowl speed were varied. The results obtained are shown in Figures 7 and 8.

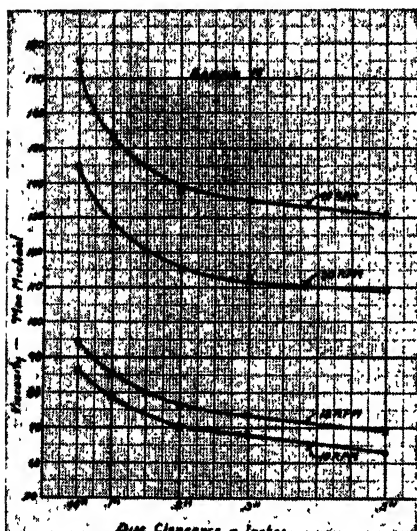


FIG. 5

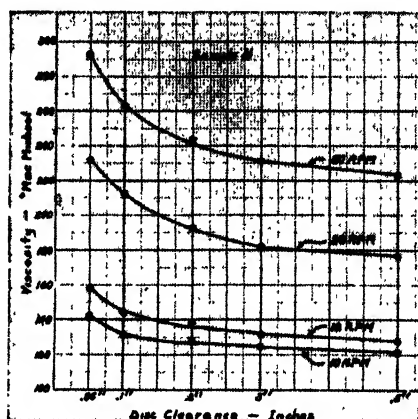


FIG. 6

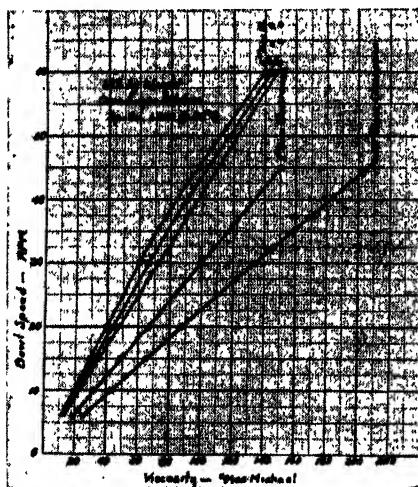


FIG. 7

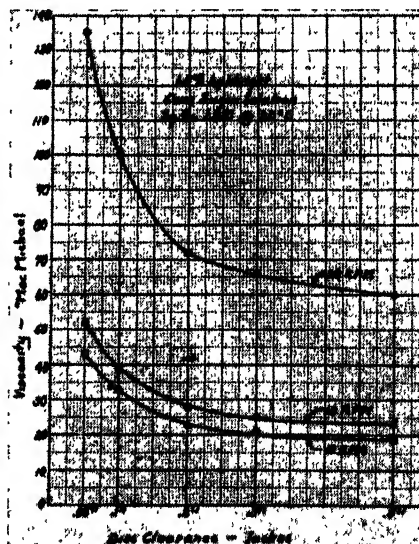


FIG. 8

From Fig. 7 it is seen that as the bowl speeds up to 20 r.p.m. the points of bowl speed *vs.* viscosity fall in a straight line, but that at bowl speeds above 20 r.p.m., some of the points are not in a straight line. All these

points should fall in a straight line because with a pure solution, such as the 60 per cent sugar solution, viscosity (not plasticity) is being measured, and the resistance to flow is directly proportional to the force applied. However, it is seen from Fig. 7 that at some of the bowl speeds above 20 r.p.m. the viscosity is greater than it should be, due perhaps to turbulent flows set up at the higher bowl speeds.

Some work was done in comparing five different No. 30 wires to see if there was much variation. Four new wires and one wire that had been in use for approximately one year were used. A 60 per cent sugar solution was used at 30°C., and the bowl speed varied from 12 to 60 r.p.m. The disc clearance was set at 0.5 inch. The results are shown in Table 1.

TABLE 1.—*Results showing differences in wires used*  
(60% cane sugar solution, sp. gr. 1.281 at 30°C.)

| BOWL SPEED | WIRE NO. |     |     |     |             |
|------------|----------|-----|-----|-----|-------------|
|            | 1        | 2   | 3   | 4   | ONE YR. OLD |
| r.p.m.     |          |     |     |     |             |
| 12         | 20       | 20  | 20  | 20  | 20          |
| 20         | 36       | 36  | 38  | 37  | 37          |
| 30         | 56       | 58  | 58  | 57  | 58          |
| 40         | 77       | 78  | 77  | 76  | 78          |
| 50         | 99       | 102 | 100 | 100 | 101         |
| 60         | 120      | 121 | 120 | 120 | 121         |

The results (Table 1) show that there is practically no difference in these wires. If all No. 30 wires check as closely as these, no trouble would be caused by them. It is interesting to note that when these results are plotted (bowl speed *vs.* viscosity) all the points fall in a straight line, even at 60 r.p.m. This is most likely due to the fact that the readings were taken very quickly, before turbulent flows started.

It is also shown that the viscosity of the 60 per cent sugar solution used in Table 1 is a little lower than that of Figs. 7 and 8. Both solutions had the same specific gravity (1.281 at 30°C.), but they were made up at different times from different bags of sugar. Perhaps not all pure commercial cane sugars will give the same viscosity in 60 per cent solutions.

Four cane sugars from different refineries were used to see if there would be any difference between the viscosity if these four sugars were made up to 60 per cent solutions, all with the same specific gravity.

The following directions were used:

Place 300 grams of sugar and 20 grams of distilled water in a liter flask. To the flask attach a reflux condenser, shake the contents occasionally, and heat to a boil. After allowing to boil 5 minutes extinguish the flame and allow the contents to cool. Take the specific gravity with a pycnometer and adjust to 1.281 at 30°C. either by adding or evaporating water from the solution. Check the 60 per cent sugar solutions for viscosity in the MacMichael Viscosimeter.

The results at 30°C. are given in Table 2.

TABLE 2.—*Results showing differences in sugar solutions*

| Solution No.                  | 1  | 2  | 3  | 4  | 1    | 2  | 3  | 4  |
|-------------------------------|----|----|----|----|------|----|----|----|
| r.p.m.                        | 12 | 12 | 12 | 12 | 20   | 20 | 20 | 20 |
| Viscosity Disc "as is"        | 32 | 32 | 32 | 31 | 52.5 | 53 | 52 | 52 |
| Viscosity 0.5" Disc Clearance | 23 | 23 | 23 | 23 | 37   | 37 | 37 | 38 |

The results in Table 2 indicate that any good grade of cane sugar could be used for making the 60 per cent solution.

The associate referee hoped to find answers to the following questions by collaborative work:

1. Is 12 r.p.m. a good bowl speed?
2. Do all 60 per cent sugar solutions made by collaborators have the same viscosity?
3. Would better results be obtained if a 0.5 inch disc clearance were used?

#### COLLABORATIVE WORK

The following instructions were sent out to the collaborators:

1. *60% sugar solution, using sugar which we sent you.*—(a) Boil solution 5 min., using a reflux condenser; (b) cool and let stand 5 hours; (c) check the sp. gr. at 30°C. It should be 1.281.

2. *Setting machine.*—(a) Use a No. 30 wire; (b) disc diameter—6 cm. (approx.); (c) viscosimeter bowl: diam. about 7 cm. and depth about 6 cm.; (d) set machine so that bowl makes 12, 20, 30, 40, 50, and 60 r.p.m., checking speed accurately with a stop watch.

3. *Running viscosity on 60% sugar solution.*—(a) Use 100 cc. of solution; (b) use a temp. of 30°C. (very important); (c) run the viscosity at the various bowl speeds indicated above; (d) run the viscosity with the disc clearance as you ordinarily have it (designated on the Data Sheet as "Disc Clearance As Is"); (e) run the viscosity with the disc clearance 0.5 inch; (f) report results in degrees MacMichael on the Data Sheet.

The sample of flour marked "X" is to be run for viscosity according to the following directions:

1. *Setting of machine.*—Same as previous directions except that the disc clearance should be "as is" and also at 0.5 inch.

2. *Preparation of flour suspension.*—(a) Use mortar and pestle: (1) Mortar size: Inside top diameter, 9.5 cm.; depth, 5.5 cm.; volume, 300.0 cc. (2) Pestle size: Diameter of butt, 3.5 cm. (b) Weight of flour: The viscosity is run on the basis of 2 grams of protein. Weigh sample so that 2 grams of protein will be present. (c) Method of Mixing: Place 50 cc. of water in a mortar and put the weighed sample on top of the water. Mix for 2 minutes, using the pestle. The speed of mixing is approximately 3 revolutions of the pestle per second. Pour this suspension into a 250 cc. Erlenmeyer flask. Rinse mortar and pestle with two 25 cc. portions of distilled water and pour these rinsings into the flask. This brings the total volume of water

TABLE 3.—*Collaborative results on "60% sugar solution"*  
(a) Disc Clearance "As Is"

| COLLABO-<br>RATOR | NO. 1 | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 16  | 17  | AVERAGE |
|-------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| r.p.m.            |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| 12                | 26    | 28  | 44  | 26  | 26  | 34  | 60  | 28  | 26  | 42  | 29  | 31  | 25  | 23  | 24  | 25  | 30.65   |
| 20                | 41    | 50  | 65  | 45  | 44  | 55  | 99  | 48  | 43  | 72  | 47  | 52  | 41  | 39  | 42  | 42  | 51.05   |
| 30                | 73    | 70  | 106 | 71  | 68  | 87  | 147 | 72  | 73  | 109 | 72  | 82  | 69  | 62  | 61  | 65  | 79.6    |
| 40                | 95    | 108 | 144 | 95  | 96  | 120 | 196 | 97  | 95  | 145 | 93  | 106 | 86  | 83  | 88  | 90  | 107.3   |
| 50                | 119   | 134 | 165 | 120 | 127 | 156 | 246 | 122 | 118 | 172 | 125 | 132 | 114 | 113 | 102 | 107 | 134.5   |
| 60                | 143   | 168 | 188 | 145 |     | 218 |     | 148 | 145 | 202 | 157 | 156 | 150 | 133 | 135 | 128 | 157.5   |

(b) Disc Clearance Set at 0.5 inch

| COLLABO-<br>RATOR | NO. 1 | 2   | 3   | 4   | 5  | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 16  | 17  | AVERAGE |
|-------------------|-------|-----|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| r.p.m.            |       |     |     |     |    |     |     |     |     |     |     |     |     |     |     |     |         |
| 12                | 23    | 30  | 36  | 20  | 23 | 22  | 20  | 20  | 22  | 19  | 26  | 24  | 22  | 20  | 18  | 24  | 23      |
| 20                | 39    | 47  | 50  | 36  | 37 | 40  | 36  | 36  | 38  | 38  | 42  | 48  | 35  | 33  | 34  | 38  | 39.1    |
| 30                | 64    | 70  | 80  | 56  | 60 | 62  | 58  | 56  | 59  | 63  | 70  | 78  | 54  | 52  | 59  | 57  | 62.1    |
| 40                | 86    | 100 | 109 | 77  | 86 | 82  | 80  | 76  | 80  | 84  | 93  | 100 | 81  | 72  | 81  | 76  | 85.2    |
| 50                | 113   | 132 | 138 | 99  |    | 100 | 108 | 100 | 109 | 115 | 115 | 121 | 113 | 94  | 100 | 99  | 110.2   |
| 60                | 137   | 160 | 176 | 120 |    | 123 |     | 124 | 133 | 148 | 145 | 144 | 145 | 118 | 149 | 121 | 138.8   |



used for the suspension up to 100 cc. (d) Digestions: Bring the flour suspension in the flask to 30°C. and place the flask in a thermostat at 30°C. *for one hour*. At the end of the first 30 minutes shake the flask. At the end of the hour add 9 cc. of 1 *N* lactic acid and again shake the flask. Let stand for 5 minutes and then pour the acidulated flour suspension into the bowl of the viscosimeter.

3. *Method for running the viscosity.*—(a) Stir the flour suspension in the bowl with the disc by moving the disc up and down about 15 times. (b) Start the viscosimeter motor and take the reading of the viscosity in degrees MacMichael. When the disc starts to swing, dampen the swing by placing the top of the finger on the viscosimeter pointer and gradually lower the hand so that part of the finger comes in contact with the swinging dial. When the dial comes to rest (after dampening), take the reading. (c) For each individual speed of bowl and each disc clearance, use a new flour suspension.

4. *Reporting results.*—(a) Report results in degrees MacMichael on the Data Sheet which we have supplied you. Also fill in the other information asked for on the Data Sheet.

#### DATA SHEET

##### 60% Sugar Solution (30°C.)

| SPEED (r.p.m.) | VISCOSITY "MACMICHAEL"<br>DISC CLEARANCE "As Is" | VISCOSITY "MACMICHAEL"<br>DISC CLEARANCE 0.5" |
|----------------|--|---|
| 12             |  |   |
| 20             |  |   |
| 30             |  |   |
| 40             |  |   |
| 50             |  |   |
| 60*            |  |   |

\* If you cannot get 60 r.p.m., get as close to it as possible and give speed.

Sp. grav. of sugar solution with hydrometer \_\_\_\_\_

Sp. grav. of sugar solution with pycnometer \_\_\_\_\_

NOTE: Specific gravities at 30°C.

#### FLOUR SAMPLE "X"

Report as directed above.

Diameter "D" of Disc

Thickness "T" of Disc

Weight of Disc

Take measurements with micrometer

Date \_\_\_\_\_ Readings by \_\_\_\_\_

#### KEY TO COLLABORATORS

1. Ohio Agricultural Experiment Station, E. G. Bayfield.
2. Bureau of Chemistry and Soils, L. H. Bailey.
3. Kansas Flour Mills Corp., W. L. Heald.
4. Pillsbury Flour Mills Co., Minneapolis.
5. Continental Baking Co., G. C. Robinson.
6. Pillsbury Flour Mills Co., Springfield.
7. W. E. Long Co., I. O. Juvrud.
8. F. W. Stocks and Sons, E. E. Smith.

TABLE 4.—*Collaborative results on "Flour Sample X"*  
(a) Disc Clearance "As Is"

| OSGILBY-<br>BAYNE | NO. 1 | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | AVERAGE |
|-------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| r.p.m.            |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| 12                | 113   | 110 | 110 | 114 | 103 | 123 | 119 | 122 | 109 | 132 | 112 | 115 | 112 | 110 | 122 | 103 | 120 | 114.7   |
| 20                | 138   | 142 | 136 | 135 | 130 | 154 | 160 | 143 | 132 | 162 | 135 | 142 | 143 | 131 | 125 | 127 | 147 | 140.0   |
| 30                | 167   | 158 | 182 | 163 | 153 | 178 | 193 | 164 | 156 | 190 | 163 | 165 | 165 | 154 | 164 | 152 | 165 | 166.6   |
| 40                | 185   | 172 | 194 | 185 | 180 | 205 | 224 | 191 | 177 | 211 | 186 | 180 | 188 | 172 | 184 | 178 | 188 | 188.2   |
| 50                | 207   | 205 | 211 | 205 |     | 229 | 256 | 215 | 209 | 241 | 207 | 195 | 220 | 194 | 207 | 195 | 212 | 212.8   |
| 60                | 225   | 237 | 251 | 230 |     | 250 |     | 239 | 228 | 261 | 223 | 210 | 225 | 220 | 237 | 217 | 239 | 232.7   |

(b) Disc Clearance Set at 0.5 inch

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |       |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| 12 | 110 | 95  | 106 | 106 | 95  | 114 | 110 | 96  | 107 | 113 | 110 | 110 | 110 | 102 | 115 | 101 | 112 | 106.6 |
| 20 | 132 | 108 | 124 | 123 | 117 | 151 | 131 | 118 | 122 | 134 | 127 | 132 | 140 | 121 | 128 | 124 | 137 | 127.5 |
| 30 | 159 | 125 | 147 | 146 | 134 | 163 | 158 | 140 | 150 | 156 | 152 | 143 | 147 | 142 | 157 | 149 | 157 | 148.5 |
| 40 | 174 | 150 | 175 | 169 | 153 | 184 | 175 | 164 | 173 | 178 | 174 | 168 | 180 | 165 | 177 | 168 | 183 | 171.2 |
| 50 | 193 | 180 | 184 | 188 |     | 212 | 193 | 186 | 190 | 201 | 195 | 178 | 202 | 185 | 207 | 189 | 197 | 192.4 |
| 60 | 215 | 200 |     | 208 |     | 231 |     | 207 | 213 | 227 | 213 | 188 | 225 | 212 | 212 | 210 | 224 | 213.0 |

9. University of Nebraska, M. J. Blish.
10. Commander Larabee Corp., W. L. Rainey.
11. Iglehart Bros. Inc., H. G. Walter.
12. Pillsbury Flour Mills Co., Astoria.
13. Mid-West Laboratories (For Wallace & Tiernan Co.), H. M. Simons.
14. The Independent Biscuit Mfg. Co., R. Bohn.
15. Michigan Bakeries Inc., R. J. Clark.
16. United Mills Co., W. E. Brownlee.
17. The Kroger Food Foundation, G. Garnatz.

### RESULTS ON COLLABORATIVE WORK

Table 3 shows the results on the 60 per cent sugar solution with disc clearance "As Is" and at 0.5 inch. It is noted that if curves, which should be straight lines, were drawn for the results on this table at the higher speeds the viscosity would actually be higher than it should be. This is most likely due to turbulent flows which are set up at the higher speeds. This, therefore, would discourage the use of higher speeds.

Table 4 shows results on Flour "X" with disc clearance "As Is" and at 0.5 inch. Table 5 was prepared in the following manner: If it is assumed that a normal No. 30 wire gives the following results with a 60 per cent sugar solution at 30°C. and a disc clearance of 0.5 inch:

|           |    |    |    |    |    |      |
|-----------|----|----|----|----|----|------|
| R.P.M.    | 12 | 20 | 30 | 40 | 50 | 60   |
| Viscosity | 20 | 37 | 58 | 78 | 99 | 120, |

plotting the above results produces a line that has a slope of .96. Assume now that some collaborator's No. 30 wire is not the same as a normal No. 30 wire. Plotting his results on the 60 per cent sugar solution at 30°C. and 0.5 inch disc clearance would give a different slope than the normal wire. (Only the first three or four speeds were used in most cases (12, 20, and 30 r.p.m.) for plotting these lines as at the higher speeds erroneous results were obtained.) Suppose the collaborator's wire gives results with a slope of 0.9. Then using the ratio of 0.9/96 and multiplying his results on Flour "X" at 0.5 inch disc clearance by this ratio he would get his results the same as if he had used a normal wire.

Table 6 shows the standard deviations and per cent deviations.

TABLE 5.—*Collaborative results on "Flour X" Correcting for Slope of 60% Sugar Line*

| COLLABORATOR | NO. 1 | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | AVERAGE |
|--------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| r.p.m.       |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| 12           | 107   | 91  | 95  | 106 | 91  | 111 | 110 | 97  | 105 | 103 | 100 | 100 | 110 | 112 | 110 | 113 | 116 | 104.6   |
| 20           | 127   | 103 | 112 | 123 | 112 | 147 | 131 | 120 | 119 | 122 | 115 | 120 | 140 | 133 | 122 | 139 | 141 | 125.1   |
| 30           | 154   | 120 | 137 | 146 | 129 | 158 | 158 | 142 | 146 | 142 | 138 | 130 | 147 | 156 | 150 | 167 | 162 | 146.0   |
| 40           | 189   | 143 | 157 | 169 | 146 | 179 | 175 | 166 | 169 | 162 | 158 | 152 | 180 | 181 | 169 | 188 | 189 | 167.7   |
| 50           | 187   | 172 | 166 | 188 |     | 207 | 193 | 188 | 185 | 181 | 177 | 161 | 202 | 203 | 198 | 212 | 203 | 188.8   |
| 60           | 207   | 192 |     | 208 |     | 224 |     | 210 | 208 | 207 | 193 | 170 | 225 | 233 | 203 | 235 | 231 | 210.3   |

TABLE 6.—*Standard and per cent deviations on collaborative results*

| R.P.M.             | 60% SUGAR<br>"AS IS" | 60% SUGAR<br>0.5" DISC<br>CLEARANCE | FLOUR X<br>"AS IS" | FLOUR X<br>0.5" DISC<br>CLEARANCE | FLOUR X<br>CORRECTED FOR<br>SLOPE OF 60%<br>SUGAR LINE |
|--------------------|----------------------|-------------------------------------|--------------------|-----------------------------------|--|
| Standard Deviation |                      |                                     |                    |                                   |  |
| 12                 | 9.38                 | 4.69                                | 6.63               | 6.08                              | 6.245  |
| 20                 | 14.83                | 4.896                               | 11.83              | 10.68                             | 14.83  |
| 30                 | 21.19                | 8.0                                 | 12.04              | 9.38                              | 10.68  |
| 40                 | 28.49                | 9.59                                | 13.82              | 8.72                              | 11.7   |
| 50                 | 34.26                | 12.08                               | 18.63              | 11.09                             | 16.26  |
| 60                 |                      | 16.88                               | 14.18              | 13.82                             | 19.34  |
| Per Cent Deviation |                      |                                     |                    |                                   |  |
| 12                 | 32.68                | 20.4                                | 5.78               | 5.7                               | 5.97   |
| 20                 | 29.05                | 12.5                                | 8.45               | 8.38                              | 11.55  |
| 30                 | 21.19                | 12.9                                | 7.23               | 6.31                              | 7.31   |
| 40                 | 28.49                | 11.26                               | 7.35               | 5.09                              | 6.98   |
| 50                 | 34.26                | 10.98                               | 8.75               | 5.77                              | 8.62   |
| 60                 |                      | 12.17                               | 6.095              | 6.49                              | 9.19   |

## DISCUSSION OF COLLABORATIVE WORK

Judged from the results in Table 6, the Per Cent Deviations of the Flour Sample "X," it might be concluded that a bowl speed of 12 r.p.m. was most satisfactory. However, this is not the case with the sugar solution. It may be possible that all the 60 per cent sugar solutions made by the collaborators did not have the same viscosity. It is also noted that the Per Cent Deviations for the sugar solutions are more than twice the amount of the flour sample, tending to show that the various sugar solutions have different viscosities.

A Disc Clearance of 0.5 inch appears to be better than the "As Is" Clearance. This shows the possibility that all the "As Is" Disc Clearances are not the same.

In correcting for the slope of the line when plotting Bowl Speed *vs.* Viscosity of the 60 per cent sugar solutions the results are no better. The explanation of this most likely is that all the 60 per cent sugar solutions were not of the same viscosity. Because the associate referee believed that all the 60 per cent sugar solutions were not of the same viscosity and that the "As Is" Disc Clearances of the collaborators were not the same, he carried out additional collaborative work as follows:

## SECOND COLLABORATIVE WORK

A sample of flour ("Y") and a 60 per cent sugar solution prepared in this laboratory were sent to the various collaborators. The method of

TABLE 7.—*Second collaborative results*  
(a) Disc Clearance "As Is" (60% Sugar Solution 30°C.)

| COLLABORATOR  | 1  | 2  | 3  | 4  | 5  | 6  | 7   | 9  | 10 | 11 | 12 | 14 | 17 | AVERAGE |
|---------------|----|----|----|----|----|----|-----|----|----|----|----|----|----|---------|
| <i>r.p.m.</i> |    |    |    |    |    |    |     |    |    |    |    |    |    |         |
| 12            | 25 | 22 | 31 | 31 | 26 | 23 | 60  | 21 | 23 | 20 | 25 | 25 | 24 | 27.38   |
| 20            | 41 | 38 | 40 | 52 | 45 | 39 | 100 | 39 | 46 | 34 | 44 | 42 | 40 | 46.15   |
| 30            | 64 | 57 | 60 | 82 | 67 | 60 | 148 | 58 | 69 | 54 | 67 | 64 | 64 | 70.30   |

(b) Disc Clearance 0.5 inch (60% Sugar Solution 30°C.)

|    |    |    |    |    |    |    |    |    |    |    |    |    |    |       |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-------|
| 12 | 21 | 23 | 24 | 23 | 20 | 23 | 21 | 17 | 20 | 19 | 20 | 20 | 22 | 21    |
| 20 | 35 | 37 | 39 | 38 | 36 | 36 | 37 | 32 | 35 | 33 | 40 | 33 | 38 | 36.07 |
| 30 | 57 | 57 | 58 | 62 | 57 | 57 | 59 | 56 | 56 | 51 | 61 | 52 | 56 | 56.84 |

(c) Flour Sample "Y"

|                    |     |    |     |     |     |     |     |     |     |     |     |     |     |        |
|--------------------|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|
| <i>Disc Clear.</i> |     |    |     |     |     |     |     |     |     |     |     |     |     |        |
| "As Is"            | 115 | 96 | 116 | 115 | 107 | 110 | 140 | 105 | 107 | 121 | 117 | 115 | 115 | 113.76 |
| 0.5"               | 112 | 88 | 102 | 106 | 93  | 107 | 107 | 91  | 101 | 121 | 107 | 104 | 108 | 103.61 |

(d) Disc Clearance ("As Is")

|   |      |      |      |      |      |      |      |      |      |      |      |      |
|---|------|------|------|------|------|------|------|------|------|------|------|------|
| X | .378 | .243 | .130 | .102 | .359 | .035 | .375 | .161 | .234 | .269 | .166 | .175 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|

running the viscosity was the same as that requested in the previous collaborative work, with the exception that the 60 per cent sugar solution was run at only 3 bowl speeds: 12, 20 and 30 r.p.m. The flour was run at only one bowl speed, 12 r.p.m.

By use of a depth gage and a micrometer, both reading to one thousandth of an inch, the "As Is" Disc Clearance was also to be determined.

Tables 7a, b, c, and d, and 8 give the results of the collaborators.

TABLE 8.—*Standard and per cent deviations on second collaborative results*

| R.P.M.             | 60% SUGAR<br>"AS IS" | 60% SUGAR<br>0.5" DISC<br>CLEARANCE | FLOUR Y<br>"AS IS" | FLOUR Y<br>0.5" DISC<br>CLEARANCE | FLOUR Y<br>CORRECTED FOR<br>SLOPE OF 60%<br>SUGAR LINE |
|--------------------|----------------------|-------------------------------------|--------------------|-----------------------------------|--|
| Standard Deviation |                      |                                     |                    |                                   |  |
| 12                 | 9.95                 | 1.87                                | 9.95               | 8.61                              | 15.06  |
| 20                 | 16.14                | 2.45                                |                    |                                   |  |
| 30                 | 23.43                | 3.16                                |                    |                                   |  |
| Per Cent Deviation |                      |                                     |                    |                                   |  |
| 12                 | 36.3                 | 8.9                                 | 8.75               | 8.31                              | 14.46  |
| 20                 | 44.7                 | 6.8                                 |                    |                                   |  |
| 30                 | 41.2                 | 5.56                                |                    |                                   |  |

#### DISCUSSION OF SECOND COLLABORATIVE WORK

In Table 8 it is noted that the 0.5 Inch Disc Clearance gives better results than the "As Is" Disc Clearance, both in the flour and the 60 per cent sugar solution. This is partially explained by Table 7, which shows that the "As Is" Disc Clearance of the various collaborators varies considerably. Figs. 4, 5, and 6 show what effect such variations of disc clearances would have upon viscosity.

It is also interesting to note that the Per Cent Deviation of the 60 per cent sugar solution at the 0.5 Inch Disc Clearance, made up in this laboratory and sent to various collaborators, checks quite closely with the flour sample that was sent out. However, in Table 6 it is noted that at the 0.5 Inch Disc Clearance the Per Cent Deviation of the 60 per cent sugar solution is considerably more than the Per Cent Deviation of the flour, leading to the belief that all the 60 per cent sugar solutions made up by the collaborators were not of the same viscosity. From Table 8 it is also noted that when the flour viscosity at the 0.5 Inch Disc Clearance is corrected for the slope of the 60 per cent sugar line at 0.5 Inch Disc Clearance, the Standard Deviation and the Per Cent Deviation are much greater than the Standard and Per Cent Deviations of the Flour "Y"

at 0.5 Inch Disc Clearance. Such results would indicate that some error must have been made in taking the readings of the 60 per cent sugar solution at 0.5 Inch Disc Clearance, or that the solution was not at exactly 30°C. For instance, a variation of only 1° or 2° MacMichael in viscosity of the 60 per cent sugar solution at 20 r.p.m. would change the slope of the line considerably and would therefore increase an error when correcting the flour viscosity for the slope of this line. The result of one collaborator checked very closely with the average of a 60 per cent sugar solution and the Disc Clearance at 0.5 inch. However, his viscosities on the flour sample "Y" were considerably lower than the average. When this collaborator re-checked his viscosity values on the flour sample "Y" his results checked very closely with the average of the viscosity on the flour sample "Y." This shows that there is still some error either in taking the readings or making up the flour water suspension.

It is also interesting to note in comparing Tables 6 and 8 with a table in last year's report, *This Journal*, 18, 577 (1936), that the Per Cent Deviations of the flour with the Disc Clearance "As Is" are much better when the 9 cc. of lactic acid was added all at once than when it was added in 2 cc. aliquots. It would therefore seem that if only the final viscosity reading is to be used, better results would be obtained if the acid were added all at once. It is known that after the suspension is acidulated continuous stirring or shaking of the suspension will lower the results.

#### SUMMARY

1. Considering the results of Tables 6, 7(d), and 8, the associate referee recommends a disc clearance of 0.5 inch. (If it is impossible to conveniently obtain a 0.5 inch disc clearance on all the MacMichael viscosimeters now used for flour viscosities, a 0.25 inch disc clearance (to be established by micrometric measurements) is suggested.)

2. From the results shown in Tables 6 and 8 he would conclude that not all collaborators are getting 60 per cent sugar solutions of the same viscosity, and that a bowl speed of 12 r.p.m., which is the common usage at present, should be retained.

3. Until better checks upon the 60 per cent sugar solutions are possible, he cannot recommend making a correction for the slope of the 60 per cent sugar solution upon the flour viscosity.

#### ACKNOWLEDGMENT

This report was made possible through the courteous and prompt support of the collaborators, and the associate referee desires that due credit be given them. He also thanks the Eimer and Amend Company for their support and loan of certain equipment necessary in conducting this work.

Walter Tholstrup presented many valuable suggestions and conducted several of the determinations presented in this report as well as supervised

the sending out of the collaborative work, and to him heartiest appreciation is extended.

No report on cold water extract flour was given by the associate referee.

## REPORT ON ERGOT IN FLOUR

By CLINTON L. BROOKE (Pillsbury Flour Mills Co.,  
Minneapolis, Minn.), *Associate Referee*

No collaborative work was done during the past year, as the results obtained by the associate referee were not particularly encouraging. It has been found that the quantitative extraction and determination of the minute quantities of alkaloid contained in even the maximum amounts of ergot present in American commercial flours is a difficult and elusive problem. These difficulties are no doubt due to the instability of the alkaloids when mixed with flour. It is hoped that during the coming year collaborative work can be invited on an accurate method for the detection and determination of the pigments of ergot, since these apparently are fairly stable.

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For report on catalase and proteolytic enzymes, see report of the Associate Referee on Enzymes, *This Journal*, 19, 372 (1936).

## REPORT ON COLOR IN FLOUR

By H. K. PARKER (Wallace and Tiernan Co., Inc.,  
Newark, N. J.), *Associate Referee*

In accordance with the recommendation in the first report on color in flour, *This Journal*, 18, 593 (1935), studies were made to develop a method to prepare a flour and water dough that would give reproducible results that correspond to bread crumb color. These studies must be continued as some variables that influence dough color have become more evident than they were in the preliminary work. It is hoped that these factors can be brought under control, and more work to this end is proposed for the coming year. Apparently temperature of water, degree of water absorption, time of mixing, and method of mixing play important rôles. Jago<sup>1</sup> describes some of these difficulties and proposes a method of mixing followed by reading the dough color through a colorless glass in order to prevent surface change due to the exposure to the air.

Further data have been accumulated in the hope of finding better correlation of the various colors to flour characteristics. These data tend to

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<sup>1</sup> *Technique of Bread Making*, p. 492, 1921. Ed. Northern Pub. Co., Liverpool.



confirm those reported by Baker, Parker and Freese.<sup>1</sup> No tabulation will be attempted at this time, and it will perhaps suffice to say that there is no more correlation between yellow in flour and flour ash as measured by this method of reflectance than Coleman and Christie<sup>2</sup> found in their extraction studies. There is some evidence that correlation between red color readings and ash exists. The black to ash correlation is less satisfactory, while the sum of the red and black readings to ash seems somewhat interesting. The correlations improve when extracted flours are studied, indicating again the importance of this method of flour color study, since the constituents associated with the red and black readings of the reflection method are not considered or evaluated by the older extraction method.

In order to carry out the studies of color readings of the same sample by different observers in different laboratories, eight N-A Colorimeters were sent out to a corresponding number of laboratories that had signified their willingness to collaborate in the test (see end of report). At various intervals samples of the same flour were sent with the instruction to the various observers that the colorimeter determination be made as nearly as possible on the same day. The samples were sent in sealed Mason jars to prevent loss of moisture and possible change of color due to dirt and dust contamination or oxidation.

The first two samples were different flours and were intended for practice determinations, while the third and fourth samples were the same flour. The purpose of the latter experiments was to learn whether or not the different observers could check themselves when using the same flour. To prevent any change occurring in the flour for test No. 4, it was stored in an electrical refrigerator in the dark at about 0°C. The results of these tests confirm the findings of Ferrari and Bailey<sup>3</sup> and indicate that this technic is permissible, for no change was observed in the flour stored under these conditions for 30 days.

In general, the first two tests showed some agreement among the observers, but it was not especially close. Some observers checked others very well. It was thought that possibly those out of line might be making readings under adverse lighting conditions, so in the second series of tests a darkened room or a dark room for reading was specified. Strong side lights changed the readings considerably; for example, a strong light from the left caused the black reading to diminish while the yellow reading increased. If, on the other hand, the strong light came from the right, the reading differences were reversed so that the black reading increased, etc. Furthermore, it seemed evident that there occurred the same phenomenon that has sometimes been noted in this laboratory; namely, that not all observers select the same hue combination to match the sample.

<sup>1</sup> *Cereal Chem.*, 10, 437 (1933).

<sup>2</sup> *Ibid.*, 3, 188 (1926).

<sup>3</sup> *Ibid.*, 6, 460 (1929).

It appears that some observers read consistently higher red values than the average. This seems to be a function of the individual eye for, from experience in this laboratory, it is noted that any combination that seems to be a perfect match to one observer appears reddish to another. It is of interest to report an observer who cannot match left and right eye readings, the right eye match appearing reddish in cast to the left eye. This finding tends to confirm Jago's<sup>1</sup> experience with the Lovibond instrument in the measurement of flour color, for he found that results among various observers were not concordant.

TABLE 1.—*N-A colorimeter study—a family flour*  
(Average of 10 readings by 17 different observers in nine different laboratories)

| LAB.                | OBSERVER NO. | TEST NO. 3 |       |       |       | TEST NO. 4 |       |       |       |
|---------------------|--------------|------------|-------|-------|-------|------------|-------|-------|-------|
|                     |              | YELLOW     | RED   | BLACK | WHITE | YELLOW     | RED   | BLACK | WHITE |
| A                   | 1            | 21.55      | 7.93  | 5.34  | 65.17 | 21.37      | 7.82  | 5.07  | 65.74 |
|                     | 4            | 24.47      | 7.94  | 5.19  | 62.40 | 22.99      | 7.32  | 5.09  | 64.60 |
| B*                  | 2            | 24.00      | 7.00  | 2.10  | 66.90 | 24.80      | 8.60  | 4.80  | 61.80 |
|                     | 3            | 24.60      | 7.00  | 2.05  | 66.35 | 23.80      | 7.70  | 4.70  | 63.80 |
| C                   | 5            | 23.70      | 6.50  | 6.50  | 63.30 | 20.85      | 10.10 | 3.90  | 65.15 |
|                     | 6            | 22.30      | 7.70  | 5.80  | 64.20 | 21.08      | 9.60  | 3.75  | 59.87 |
|                     | 16           | 22.60      | 7.60  | 6.60  | 63.20 | 20.00      | 9.45  | 4.30  | 66.25 |
| D                   | 7            | 21.55      | 8.45  | 3.10  | 66.90 | —          | —     | —     | —     |
|                     | 8            | 22.70      | 7.70  | 3.92  | 65.68 | 23.20      | 7.20  | 2.70  | 66.90 |
| E                   | 15           | 21.75      | 8.90  | 7.78  | 61.57 | 21.75      | 8.05  | 7.20  | 63.00 |
| F                   | 10           | 23.45      | 7.80  | 5.20  | 63.55 | 25.85      | 5.90  | 5.50  | 62.75 |
| G                   | 11           | 22.60      | 8.88  | 2.87  | 65.65 | 20.93      | 8.37  | 2.40  | 68.30 |
|                     | 14           | 21.79      | 8.74  | 2.37  | 67.10 | 21.56      | 7.26  | 2.72  | 68.46 |
| H                   | 12           | 21.10      | 10.10 | 7.40  | 61.50 | 20.35      | 9.20  | 7.20  | 63.25 |
|                     | 13           | 20.00      | 7.30  | 8.30  | 64.30 | 20.78      | 6.70  | 6.53  | 66.00 |
| M                   | 9            | 22.08      | 7.15  | 5.04  | 65.69 | 23.02      | 6.88  | 4.92  | 65.18 |
|                     | 17†          | —          | —     | —     | —     | 22.46      | 7.64  | 5.00  | 64.90 |
| Average of Averages |              | 22.52      | 7.92  | 4.97  | 64.59 | 22.17      | 7.99  | 5.09  | 64.75 |

\* B's No. 3 Test ran with old discs. No. 4 Test ran with new discs.

† Average of five readings.

A study of the results of the 3rd and 4th collaborative tests, Table 1, shows the same sort of findings among observers, and that some checked themselves well while others did not. In the case of two laboratories, it was found that the discs had become soiled and darkened, so that when collaborators were supplied with a new set of discs different readings were

<sup>1</sup> Loc. cit.

obtained. This observation emphasized the fact that the discs must be kept scrupulously clean and covered when not in use, but it throws some doubt upon the validity of making comparisons of much value between the results of No. 3 and No. 4. In other words, to be able more accurately to record the variation between the individual eyes, it seems necessary to be assured that reading conditions are the same. The most important of these conditions are absolutely clean (preferably new) discs, no side lights, and the same sample.

Some collaborators suggested that the wet pekarized flour is not a fair test, since some variable may play a rôle in making the surface ready for measurement. The point is well taken, because it is significant that a rigid procedure in preparing the wet Pekar is vital to comparable results.

In conclusion, it seems that while the method of study of flour color by means of Maxwell discs is of great value as a research tool, it has limitations when one observer attempts to match the findings of another. Davis<sup>1</sup> makes this interesting statement in comparing photoelectric with visual brightness testers for paper: "The optical problem would be complicated by the fact that, for equivalent accuracy from operator to operator, the eyes of operators would have to be selected for suitable color sensitivity almost as carefully as the photo cells have had to be." It is believed that this inconsistency is caused by differences in the eyes of various observers or in psychological response.

### RECOMMENDATIONS\*

It is recommended—

(1) That more work be done to establish differences in observers under rigidly controlled conditions and to learn whether these observers can check themselves.

(2) That samples of some sort, such as bread, which can be read at once without encountering variables as found in the Pekar procedure, be studied collaboratively.

(3) That further studies to establish a rapid and rigid procedure for the preparation of flour and water doughs in correlation to bread crumb color be carried out.

(4) That more data be accumulated for the correlation of red to ash, black to ash, and the sum of the red and black readings to ash.

(5) That a definite standard sample be sent to collaborators periodically in order to check their discs and lighting conditions, and to help train their eyes.

### COLLABORATORS

A. Novadel-Agene Corp., Newark, N. J.

H. K. Parker No. 1 and R. Van Burek No. 4.

<sup>1</sup> *Paper Trade J.* (Tech. Assoc. Section), 101, 44 (1935).

<sup>2</sup> For report of Subcommittee C and action of the Association, see *This Journal*, 19, 65 (1936).

- B. Continental Baking Co., Jamaica, N. Y.  
E. L. Von Eschen, K. H. Lorenz, and John L. Cunningham.
- C. Continental Baking Co., Kansas City, Mo.  
Wm. Green and Coworkers.
- D. Prefers to remain unidentified.
- E. Baker's Weekly, New York, N. Y.  
Chas. Glabau and Mrs. Kepes.
- F. U. S. Bur. Agricultural Economics.  
C. C. Fifield.
- G. Igleheart Bros., Evansville, Ind.  
H. G. Walter, Noel Knight, and Grant Percy.
- H. Kroger Food Foundation, Cincinnati, Ohio.  
Geo. Garnatz, Mr. Reiman, and Mr. Loving.
- M. State Mill & Elevator Co. Grand Forks, N. H.  
L. H. Patten, Jr. and Stewart White.

## THIRD DAY

### WEDNESDAY—MORNING SESSION

#### REPORT ON FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station,  
Burlington, Vt.), *Referee*

The chapter on Grain and Stock Feeds in the 1930 edition of *Methods of Analysis* gives many official and tentative methods, which have required the study of the referees and collaborators for many years. Thousands of tests have been made with the hope that the results thus obtained may be as accurate as it is humanly possible to make them. Yet a careful study of some of the work reveals that there is not always the agreement among the chemists that there should be when they use the same sample.

Many of the minute details of the methods have not been observed. Some chemists seem to have their own ideas as to how the determination should be done—crude fiber boiling made in open beakers, protein digestions completed in 20 minutes, moisture determinations made by drying overnight in an air oven that varies in temperature, and many other digressions.

Some State feed control laws require that A.O.A.C. methods be used. If they are not used, they should be. The referee feels that all feed control and feed manufacturing chemists should adhere strictly to the official methods so that closer agreements may be obtained.

Heads of departments should keep well informed on the official changes in methods and instruct their assistants to perform accurately all their minute details.

It has been suggested that a study of a method for the determination of calcium citrate in feed be taken up by the feeding stuffs section. The determination of citric acid in cheese in our official methods and Hartmann's method for the determination of milk solids in bread has been proposed. These studies should be assigned to associate referees.

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No report on stock feed adulteration was given by the associate referee.

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#### REPORT ON MINERAL MIXED FEEDS

By H. A. HALVORSON (*Associate Referee*), and A. O. OLSON  
(Feed Laboratory, Department of Agriculture, Dairy  
and Food, St. Paul, Minnesota)

This year's work includes: (1) revision of the tentative method,<sup>1</sup> (2) improvement in the technic, (3) presentation of the procedure in greater

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<sup>1</sup> *Methods of Analysis*, A.O.A.C., 1930, 287, 3.

detail, and (4) an investigation of several factors that are believed to influence the results of calcium oxide determinations in mineral mixtures.

The present tentative method appears to be objectionable on the following grounds: (1) that low results are obtained on samples containing phosphorus, because the precipitation of the calcium oxalate is made at a hydrogen-ion concentration that allows the co-precipitation of calcium as phosphate; (2) that the action of potassium permanganate on the filter paper produces high results; (3) that calcium oxalate is slightly soluble in the wash water; and (4) that magnesium and iron sometimes present in mineral mixtures interfere with an accurate determination when calcium oxalate is precipitated in a weak acid solution or by long delayed filtration of the precipitate. Meloche and associates, *This Journal*, 16, 240 (1933), offer a procedure for the determination of calcium that is somewhat longer and more detailed than the tentative method, but one intended to overcome the inaccuracies of the latter. For convenience their procedure will be called the proposed method.

In 1933 and again in 1934, *This Journal*, 17, 173 (1934); 18, 335 (1935), the tentative and proposed methods were compared collaboratively on representative mineral mixtures, and Table 1 shows that there are no significant differences in the respective averages of the collaborators' results.

TABLE 1.—Averages of collaborators' results on 1933 and 1934 A.O.A.C. samples  
(Results expressed in percentage of CaO)

| METHOD                              | NO. 2<br>1933 | NO. 3<br>1933 | NO. 1<br>1934 | NO. 2<br>1934 |
|-------------------------------------|---------------|---------------|---------------|---------------|
| Gravimetric*                        | 42.16         | 31.42         |               |               |
| Tentative†                          | 42.24 (17)    | 31.46 (18)    | 10.42 (21)    | 51.77 (20)    |
| Proposed†                           | 42.01 (12)    | 31.38 (13)    | 10.34 (20)    | 51.70 (19)    |
| Differences Tenta-<br>tive-Proposed | +0.23         | +0.08         | +0.08         | +0.07         |

\* Reported by Griem and Clifton.

† Figures in parentheses refer to number of collaborators reporting.

Comparisons of individual results by both methods on Samples 2 and 3 (1933 report) with the amounts of calcium oxide obtained by the gravimetric method present average variations (Table 2). The upper half of the table includes the more erratic as well as the more consistent results. The lower half shows the average variations when the highest and the lowest results obtained by each method are omitted.

It will be noted that the averages (Table 1) obtained from the results of a large number of analysts using the two methods, as well as the average deviations from the calcium content of two samples by the gravi-

TABLE 2.—Average of collaborators' variations above and below gravimetric results\* on 1933 samples

(Results expressed in percentage)

| SAMPLE NO. | NUMBER OF COLLABORATORS | TENTATIVE METHOD | NUMBER OF COLLABORATORS | PROPOSED METHOD |
|------------|-------------------------|------------------|-------------------------|-----------------|
| 2          | 5                       | +0.54            | 6                       | +0.31           |
|            | 7                       | -0.35            | 6                       | -0.60           |
| 3          | 6                       | +0.50            | 5                       | +0.42           |
|            | 7                       | -0.32            | 8                       | -0.33           |
| 2          | 4                       | +0.38            | 5                       | +0.17           |
|            | 6                       | -0.32            | 5                       | -0.38           |
| 3          | 5                       | +0.34            | 4                       | +0.14           |
|            | 6                       | -0.22            | 7                       | -0.18           |

\* No. 2 = 42.16 % CaO; No. 3 = 31.42 % CaO.

metric method, indicate that the proposed method has no decided advantage over the tentative from the standpoint of accuracy as judged from analyses of products representing a variety of mineral feed mixtures. When individual results in 1934 are examined, it is found that great variations from the averages are displayed by both methods. The high and low extremes of Sample 1 by the tentative method are +0.91 and -0.82; by the proposed method, +1.18 and -0.98. The greatest deviations of Sample 2 are: tentative +1.16 to -0.71; proposed +1.58 to -0.98. These large variations may be attributed in order of their importance to several factors: first, poor technic; second, heterogeneous samples; third, faults inherent in the method. The first will account for most errors, while the second may be of considerable importance in certain cases.

The use of averages (calculated from results obtained by the method under review) as a means to approximate the correct calcium oxide content of mineral feed samples is also subject to criticism. Doubtless, a percentage nearer the true figure would be obtained if results varying greatly

TABLE 3.—Departures from reported averages\* for 1934 A.O.A.C. samples  
(Results expressed in percentage)

| LABORATORY | SAMPLE 1  |          | SAMPLE 2  |          |
|------------|-----------|----------|-----------|----------|
|            | TENTATIVE | PROPOSED | TENTATIVE | PROPOSED |
| 1          | -0.39     | -0.29    | +0.02     | +0.11    |
| 2          | -0.49     | -0.41    | -0.16     | -0.28    |
| 3          | -0.42     | -0.26    | -0.67     | -0.30    |

\* CaO by tentative, Sample 1 = 10.42 %, Sample 2 = 51.77 %; by proposed, Sample 1 = 10.34 %, Sample 2 = 51.70 %.

from the mean were omitted in calculating the accepted average. In Table 3 the results on 1934 A.O.A.C. Samples 1 and 2 reported from three laboratories, which have had considerable experience with calcium oxide determinations, are compared with the averages of the results reported by all collaborators by the tentative and proposed methods.

Omitting from consideration the report on Sample 2 by Laboratory 3, tentative method, it will be noted that the three laboratories have analyzed the samples by two methods, whose results agree closely with the general average. The conclusion appears warranted, therefore, that satisfactory results for control work can be obtained with either method.

Table 4 is presented to show the great differences in results secured on aliquots from separate weighings compared with those from aliquots taken from the same weighing. This discrepancy in results from different

TABLE 4.—*Calcium oxide obtained by tentative method from different weighings of 1933 A.O.A.C. samples*

(Results expressed in percentage)

| SAMPLE NO.  | WEIGHINGS |       |        |       |        |       |        |       |
|---|-----------|-------|--------|-------|--------|-------|--------|-------|
|   | FIRST     |       | SECOND |       | THIRD  |       | FOURTH |       |
|   | ALIQOT    | AV.   | ALIQOT | AV.   | ALIQOT | AV.   | ALIQOT | AV.   |
| 2*  | 41.70     | 41.67 | 42.35  | 42.40 | 42.62  | 42.67 | 41.84  | 41.83 |
|   | 41.63     |       | 42.45  |       | 42.72  |       | 41.91  |       |
|   |           |       |        |       |        |       | 41.73  |       |
| Max. Dif-<br>ference  | 0.07      |       | 0.10   |       | 0.10   |       | 0.18   |       |
| Maximum difference of averages from the 4 weighings— $42.67 - 41.67 = 1.00\%$ |           |       |        |       |        |       |        |       |
| 3   | 31.56     | 31.67 | 31.84  | 31.97 | 31.87  | 31.83 | 31.41  | 31.46 |
|   | 31.82     |       | 32.10  |       | 31.79  |       | 31.55  |       |
|   |           |       |        |       |        |       | 31.41  |       |
| Max. Dif-<br>ference  | 0.26      |       | 0.26   |       | 0.08   |       | 0.14   |       |
| Maximum difference of averages from the 4 weighings— $31.97 - 31.46 = 0.51\%$ |           |       |        |       |        |       |        |       |

\* For the cause of the uneven nature of this sample see *This Journal*, 17, 174 (1934).

weighings of the same product in an individual laboratory predicts variations that might be expected in reports from many laboratories working on separate portions of products containing several immiscible substances. Sample 2 consisted of a mixture of 40 per cent tribasic calcium phosphate, 40 per cent calcium carbonate, 19.95 per cent salt, and 0.05 per cent potassium iodide; Sample 3 contained 10 per cent digester tankage, 10 per cent charcoal, 25 per cent spent bone black, 35 per cent ground limestone, 19.9 per cent salt and 0.1 per cent potassium iodide.



In Table 2 of their paper, Meloche and co-workers show the errors in results secured by employment of the tentative and proposed methods on liquids made by taking definite aliquots of a standard solution of pure calcite, both alone and mixed with solutions of other salts, for the purpose of introducing known quantities of such interfering substances as phosphates, magnesium, and iron. In the trials made, the errors due to lack of uniformity of the sample and the presence of many other substances that may be found in mineral mixed feeds were not taken into account. In Table 5 the data given in Meloche's Table 2 have been arranged to show the equivalent percentages of phosphoric acid ( $P_2O_5$ ), magnesium oxide and ferric oxide in the solutions and the relationships that these bear to the calcium oxide content.

It is interesting to compare the phosphoric acid contents of the specimens obtained by mixing solutions of pure chemicals, as reported in the table, with commercial mineral feed mixtures. During the period 1929-1934 inclusive, the annual average phosphoric acid content never exceeded 11.65 per cent from a total of 462 samples of commercial mineral feeds examined by the states of Wisconsin and Minnesota. In most years the average ratio between the phosphoric acid and calcium oxide con-

tents  $\left(\frac{P_2O_5}{CaO} \times 100\right)$  was below 30 per cent, and never exceeded 34 per cent.

In only one instance among 462 such samples was the individual phosphoric acid content higher than the calcium oxide; and this was on a sample of di-calcium phosphate. Referring to Table 5, it will be noted that when the ratio of phosphoric acid to calcium oxide is less than 61.17 per cent, the error by the tentative method is generally smaller than 0.22 per cent; and in cases where the ratio is less than 40 per cent, the error is negligible.

It will be observed from the part of Table 5 showing the magnesium oxide in the samples that the percentages vary from 17.90 to 35.80, and

that the ratios  $\left(\frac{MgO}{CaO} \times 100\right)$  range from 63.36 to 126.73 per cent. In

1935, ten samples of mineral mixed feeds examined at Minnesota showed a range in magnesium oxide content of 0.26 to 3.90 per cent, the average being 1.46 per cent. The ratio  $\left(\frac{MgO}{CaO} \times 100\right)$  in the ten samples va-

ried from 0.68 to 9.60 per cent and averaged 3.87 per cent. Since large amounts of magnesium in feedstuffs are seldom encountered, any errors in the methods caused by excessive amounts of that substance may be disregarded. The same statement applies with equal or greater force to a consideration of the ferric oxide content of mineral feeds. The table shows

that the errors of the proposed method due to the presence of iron are substantially as large as those of the tentative method; also that the ferric oxide content in the specimens examined ranged from 7.27 to 36.35 per cent.

TABLE 5.—*Comparison of tentative and proposed methods*

| COMPOSITION OF SAMPLE |                               |                                 | TENTATIVE METHOD |          | PROPOSED METHOD |          |
|-----------------------|-------------------------------|---------------------------------|------------------|----------|-----------------|----------|
| CaO                   | P <sub>2</sub> O <sub>5</sub> | $\frac{P_2O_5}{CaO} \times 100$ | FOUND            | ERROR    | FOUND           | ERROR    |
| per cent              | per cent                      | per cent                        | per cent         | per cent | per cent        | per cent |
| 28.25                 | 0.00                          | 0.00                            | 28.26            | +0.01    | 28.28           | +0.03    |
| 28.25                 | 5.76                          | 20.39                           | 28.20            | -0.05    | 28.24           | -0.01    |
| 28.25                 | 11.52                         | 40.78                           | 28.03            | -0.22    | 28.22           | -0.03    |
| 28.25                 | 17.28                         | 61.17                           | 28.10            | -0.15    | 28.17           | -0.08    |
| 28.25                 | 23.04                         | 81.56                           | 27.96            | -0.29    | 28.19           | -0.06    |
| 28.25                 | 34.56                         | 122.34                          | 27.82            | -0.43    | 28.23           | -0.02    |
| 28.25                 | 46.08                         | 163.12                          | 27.73            | -0.52    | 28.22           | -0.03    |
| 56.50                 | 69.12                         | 122.34                          | 55.81            | -0.69    | 56.71           | +0.21    |
| 28.25                 | 10.74                         | 38.02                           | 28.33            | +0.08    | 28.24           | -0.01    |

| CaO   | MgO   | $\frac{MgO}{CaO} \times 100$ |       |       |       |       |
|-------|-------|------------------------------|-------|-------|-------|-------|
| 28.25 | 17.90 | 63.36                        | 28.46 | +0.21 | 28.36 | +0.11 |
| 28.25 | 25.10 | 88.85                        | 28.45 | +0.20 | 28.27 | +0.02 |
| 28.25 | 28.64 | 101.38                       | 28.38 | +0.13 | 28.42 | +0.17 |
| 28.25 | 35.80 | 126.73                       | 28.58 | +0.33 | 28.40 | +0.15 |

| CaO   | Fe <sub>2</sub> O <sub>3</sub> | $\frac{Fe_2O_3}{CaO} \times 100$ |       |       |       |       |
|-------|--------------------------------|----------------------------------|-------|-------|-------|-------|
| 28.25 | 7.27                           | 25.73                            | 28.21 | -0.04 | 28.35 | +0.10 |
| 28.25 | 21.81                          | 77.20                            | 27.96 | -0.29 | 28.31 | +0.06 |
| 28.25 | 36.35                          | 128.67                           | 28.00 | -0.25 | 28.51 | +0.26 |

The associate referee's attention has been called to the work of Curtis and Finkelstein<sup>1</sup> on errors caused by filter paper in titrations with permanganate. These authors conclude that filter paper uses an average of 0.33 cc. of 0.15 *N* potassium permanganate, resulting in an error of 0.70 per cent in a typical limestone containing 64 per cent calcium as calcium oxide. It is evident that these workers titrated the oxalate in the presence of the untreated filter paper, which does not correspond to the procedure in the tentative method in which the paper is thoroughly washed during filtration of the calcium oxalate before titration. McBride and Scherrer<sup>2</sup> show that if an end point permanent for 30 seconds is satisfactory, it

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 5, 318 (1933).

<sup>2</sup> *J. Am. Chem. Soc.*, 39, 928 (1917).

makes little difference what reagent impregnates the paper or if the solution is hot or cold. They treated filter papers with a number of reagents (including hot and cold water, hot and cold sulfuric acid and hot and cold ammonium hydroxide), and state that the first 25 cc. of the liquid passing through the paper removes all except negligible traces of the substances causing a reducing action on permanganate. Uncompleted work conducted in the associate referee's laboratory also indicates that filter paper as treated in the tentative method has little or no effect on the accuracy of the method. The description of the method has been altered, however, to make filtration on filter paper, asbestos, or sintered glass optional and in addition has provided for correction of results with a blank.

### SUMMARY

The studies of the current and past years appear to justify the following conclusions: (1) The largest errors with both the tentative and proposed methods have been due to faulty technic on the part of several analysts; (2) a small number of errors have been caused by and can be traced to lack of homogeneity in the ingredients comprising the samples taken for analysis; (3) both the tentative and the proposed method are satisfactory for control work on commercial mineral mixtures; (4) a revision of the tentative method and a statement of the procedure in greater detail will improve the technic of the inexperienced analyst.

The revised method has been published, *This Journal*, 19, 93 (1936).

It is recommended<sup>1</sup> that the revision submitted be substituted for the present tentative method for the determination of calcium oxide in mineral feeds.

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## REPORT ON IODINE IN MINERAL MIXED FEEDS

By H. A. HALVORSON (Feed Laboratory, Department of  
Agriculture, Dairy and Food, St. Paul, Minn.),  
*Associate Referee*

In accordance with the recommendations adopted last year, the method of Elmslie and Caldwell (proposed), *This Journal*, 18, 338 (1935), was compared with the Knapheide-Lamb procedure (tentative method), *Ibid.*, 17, 67 (1934). Collaborators were requested to determine iodine by the two methods in mineral mixtures containing added salts of iodine, and in other products that contained the element in organic combination. For comparative purposes, determinations by methods other than those being studied by the associate referee were also made.

The following tabulation gives the origin and description of samples

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 47 (1936).

to which reference by number is made in Table 1, which presents the iodine results obtained by the various methods.

| <i>Sample No.</i>        | <i>Description</i>  |
|--------------------------|---|
| 1                        | Dried plant material submitted by J. S. McHargue, Lexington, Ky. No iodine added since element is naturally present.  |
| 2                        | Identical material as Sample 1.   |
| 3                        | Crab meal furnished by Roe E. Remington and E. J. Coulson, Charleston, S. C. No iodine added since element is naturally present.  |
| 4                        | Dried haddock flesh. Same donors as of Sample 3. No iodine added since element is naturally present.  |
| 5                        | Shrimp hulls from fresh shrimp peeled and dried in laboratory. Same donors as of Sample 3. No iodine added since element is naturally present.  |
| 6                        | Commercial shrimp meal (waste from shrimp cannery). Same donors as of Sample 3. No iodine added since element is naturally present.   |
| 7, 8,<br>9, 10,<br>11    | Commercial mineral feed mixtures, each containing a large number of inorganic ingredients and a few organic products, furnished by the State of Minnesota Feed Laboratory. Chief source of iodine in each sample is added potassium iodide. |
| 12, 13,<br>14, 15,<br>16 | Commercial mineral feed mixtures, each containing a large number of inorganic ingredients and a few organic products, furnished by State of Indiana Feed Laboratory. Chief source of iodine in each sample is added potassium iodide.       |
| 17, 18,<br>19            | Commercial mineral feed mixtures, each containing a large number of inorganic ingredients and a few organic products, furnished by State of Wisconsin Feed Laboratory. Chief source of iodine is added potassium iodide.                    |

#### DISCUSSION OF RESULTS

As will be seen from Table 1, the results by the proposed method (Elmslie-Caldwell) are low more often than high, when compared with the iodine content obtained by the tentative (Knapheide-Lamb) method. Collaborators Griem and Clifcorn state that the low results they obtained by the proposed method were caused by volatilization of iodine during ignition of the samples and by the retention of the element in the charred mass. It will be noted from their tests that the greatest loss proportionally occurs when the largest sample is used.

The directions of the proposed method limit the portion taken for analysis, since it must contain 3-4 mg. of elemental iodine. This requirement necessitates the use of unwieldy samples when the iodine content is low, without providing for proper adjustment in the quantities of re-

agents employed. Generally, collaborators have restricted the size of samples to amounts that are convenient to manipulate in the method and with the reagents specified. The size of the sample taken in each case is recorded in Table 1, with the approximate content of iodine in number of milligrams.

TABLE 1.—*Iodine results of collaborators on various products*  
(Refer by number to description of samples)

| SAMPLE<br>NUMBER | SIZE OF SAMPLE<br>TAKEN IN K-L<br>AND E-C<br>METHODS | IODINE IN<br>SAMPLE—<br>APPROXIMATE | IODINE BY<br>K-L METHOD | IODINE BY<br>E-C METHOD | IODINE BY<br>MC HARGUE* | IODINE BY<br>REMINGTON &<br>COULSON† |
|------------------|--|-------------------------------------|-------------------------|-------------------------|-------------------------|--------------------------------------|
|                  | grams  | mg.                                 | per cent                | per cent                | per cent                | per cent                             |
| 1                | 10   | 0.40                                |                         | 0.0038 (1)              | 0.0040 (6)              |                                      |
| 2                | 10   | 0.40                                |                         | 0.0030 (1)              | 0.0040 (6)              |                                      |
| 3                | 10   | 0.20                                | 0.0057 (1)              | 0.0024 (1)              |                         | 0.00195 (5)                          |
| 4                | 10   | 0.30                                | 0.0019 (1)              | 0.0018 (1)              |                         | 0.00326 (5)                          |
| 5                | 10   | 0.40                                | 0.0051 (1)              | 0.0019 (1)              |                         | 0.00449 (5)                          |
| 6                | 10   | 0.20                                | 0.0032 (1)              | 0.0027 (1)              |                         | 0.00217 (5)                          |
| 7                | 10   | 3.17                                | 0.0317 (1)              | 0.0391 (1)              |                         |                                      |
| 7                | 10   | 3.17                                |                         | 0.0372 (2)              |                         |                                      |
| 8                | 10   | 0.31                                | 0.0031 (1)              | 0.0042 (1)              |                         |                                      |
| 8                | 10   | 0.31                                |                         | 0.0031 (2)              |                         |                                      |
| 9                | 10   | 1.64                                | 0.0164 (1)              | 0.0129 (1)              |                         |                                      |
| 9                | 10   | 1.64                                |                         | 0.0131 (2)              |                         |                                      |
| 10               | 10   | 1.51                                | 0.0151 (1)              | 0.0227 (1)              |                         | Iodine by                            |
| 10               | 10   | 1.51                                |                         | 0.0165 (2)              |                         | McCullagh‡                           |
| 11               | 10   | 5.46                                | 0.0546 (1)              | 0.0691 (1)              |                         | per cent                             |
| 11               | 10   | 5.46                                |                         | 0.0581 (2)              |                         |                                      |
| 12               | 5§   | 0.16                                | 0.0032 (3)              | 0.0028 (3)              |                         | 0.0039 (7)                           |
| 13               | 5  | 0.61                                | 0.0123 (3)              | 0.0115 (3)              |                         | 0.0139 (7)                           |
| 14               | 5§   | 0.05                                | 0.0010 (3)              | 0.0006 (3)              |                         | 0.0006 (7)                           |
| 15               | 5§   | 0.38                                | 0.0076 (3)              | 0.0073 (3)              |                         | 0.0105 (7)                           |
| 16               | 5  | 0.15                                | 0.0030 (3)              | 0.0035 (3)              |                         | 0.0037 (7)                           |
| 17               | 45   | 3.51                                | 0.0078 (4)              | 0.0017 (4)              |                         |                                      |
| 18               | 15   | 3.33                                | 0.0222 (4)              | 0.0177 (4)              |                         |                                      |
| 19               | 5  | 3.58                                | 0.0696 (4)              | 0.0510 (4)              |                         |                                      |

\* Method: McHargue and associates, *Ind. Eng. Chem. Anal. Ed.*, 6, 318 (1934), or *This Journal*, 18, 73 (1935).

† Method: Refer to U. S. Bureau of Fisheries Report No. 25 and Von Kolnits and Remington, *Ind. Eng. Chem. Anal. Ed.*, 5, 38 (1933).

‡ Method: McCullagh, *J. Biol. Chem.*, 107, 35 (1934).

§ Size of sample increased to 10 grams for E-C method, resulting in twice as many mg. of iodine as shown in table.

NOTE: Numbers in parentheses in table refer to collaborators as follows:

- (1) A. O. Olson, St. Paul, Minn.
- (2) H. B. McDonnell, College Park, Md.
- (3) H. R. Kraybill and G. E. Halliday, Lafayette, Ind.
- (4) W. B. Griem and L. E. Clifcorn, Madison, Wis.
- (5) R. E. Remington and E. J. Coulson, Charleston, S. C.
- (6) J. S. McHargue, Lexington, Ky.
- (7) D. Roy McCullagh, Cleveland, O.

A comparison of Olson's results by the tentative and proposed methods with those of Remington and Coulson, using their combustion method, shows that the results by the tentative method are nearer to the combustion method figures in only one case in four. The erratic results obtained on the marine products by the tentative and proposed methods may be caused by (1) incomplete destruction of organic matter due to high nitrogen content of samples (protein contents 33-90 per cent) and (2) the violent action (combustion) during fusing or charring. In contrast to the foregoing, the data on Samples 7-11, inclusive (commercial mineral mixtures), show results obtained in two different laboratories using the proposed method. These results may also be compared with those obtained by the tentative method. In these cases the evidence is not conclusive that greater loss of iodine occurs in the proposed than in the tentative method, especially with samples of the size indicated.

Kraybill and Halliday obtained similar results with both methods in three of the five samples examined. The greatest loss proportionally of iodine with the proposed method as compared with the tentative seems to occur in the samples containing small percentages of the element, but the results of McCullagh (collaborator No. 7), using his own method, agree more closely with determinations by the proposed method than with those by the tentative in two samples. Assuming the tentative method percentages to be most nearly correct for Samples 12-16, inclusive, the results reported by the proposed method show deficiencies from 0.0003 to 0.0008 per cent in four samples, and an average of 0.0005 per cent in one sample.

TABLE 2.—*Iodine in 1934 A.O.A.C. Mineral Feed Sample 1*

| COLLABORATOR'S NO.* | K-L METHOD<br>(TENTATIVE) | H-C METHOD<br>(PROPOSED) |
|---------------------|---------------------------|--------------------------|
| 7                   | 0.0303                    | 0.0303                   |
| 8                   | 0.0300                    | 0.0300                   |
| 11                  | 0.0313                    | 0.0295                   |
| 12                  | 0.0299                    | 0.0294                   |
| 13                  | 0.0307                    | 0.0304                   |
| 14                  | 0.0306                    | 0.0306                   |
| 15                  | 0.0311                    | 0.0302                   |
| 16                  | 0.0299                    | 0.0296                   |
| 21                  | 0.0312                    | 0.0307                   |
| Mean                | 0.0306                    | 0.0301                   |

\* Refer to table in *This Journal*, 18, 335 (1935).

The effect of the character of the samples on the results obtained has not been thoroughly investigated, but the individual determinations on which the reported percentages are based indicate that the composition

of the compound containing the iodine, as well as the uneven distribution of iodine salts in samples, may account for part of the difficulty in obtaining concordant results. This statement is confirmed by a comparison of the results given last year with those shown in Table 1. A.O.A.C. Sample 1 (1934) was a commercial feed mixture containing dried kelp mixed uniformly. A critical selection of the results of collaborators whose determinations were within 0.001 per cent of last year's reported averages by both the tentative and proposed methods produces Table 2.

The associate referee wishes to emphasize the need for more rapid and more convenient procedures for the determination of iodine in mineral mixtures. The Elmslie-Caldwell (proposed) method appears to be satisfactory, but further work is necessary to determine whether accurate results may be secured under all conditions. It is recommended:<sup>1</sup> (1) that the method proposed by Elmslie and Caldwell be further studied, and (2) that other simplified procedures for iodine in mineral mixed feeds be evaluated by collaborative work.

## REPORT ON MOISTURE IN FEEDING STUFFS

By G. E. GRATTAN (Department of Agriculture,  
Ottawa, Canada), *Associate Referee*

Last year the associate referee was instructed to revise and bring up to date the methods for moisture determination in feeding stuffs.

From comprehensive surveys during the past few years, it has been found that some methods are not being used, others have not been stated specifically enough to prevent various analysts from modifying them considerably, and others have been modified since the last edition of *Methods of Analysis* was published.

While the wording of the methods has been changed somewhat in an attempt to clarify certain points, no change has been made in the procedure. For example, in drying with heat under reduced pressure the method states: "Dry to constant weight at the temperature of boiling H<sub>2</sub>O under a reduced pressure not to exceed 100 mm. of mercury. . . ."

This direction should be changed to give a definite temperature of 70°C. This temperature is being used in the majority of laboratories that use the vacuum method. Also, the boiling point of water at 100 mm. of mercury is about 51°C. and a temperature of 70°C. apparently removes all the moisture. Again, it should be understood that a considerable number of feeds contain molasses or other sweetening agents and these should not be dried at temperatures above 70° under the official method for moisture under these headings. (See XXXIV, 4, p. 364, *Methods of Analysis*, 1930.)

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 47 (1936).

RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That a committee be appointed to study all methods for the determination of moisture in *Methods of Analysis* in an endeavor to have one official vacuum method for all materials and to correlate the other moisture methods as much as possible.

(2) That the work on moisture in feeding stuffs be discontinued for the present.

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REPORT ON BIOLOGICAL METHODS FOR  
ASSAY OF VITAMIN D CARRIERS

By W. B. GRIEM (Wisconsin Department of Agriculture  
and Markets, Madison, Wis.), *Associate Referee*

The associate referee did not deem it advisable to inaugurate further collaborative work on the tentative method for vitamin D assay by the preventive biological method, *This Journal*, 17, 69 (1934). He had hoped that during the year the reference cod liver oil for use with poultry would have been established in accordance with the tentative plans discussed informally last year. This oil would have been collaboratively assayed in order to accumulate information on the reference oil as well as on the method. The cost of collaborative work of this nature is considerable, and previous work, *Ibid.*, 180, has shown that good agreement is obtained between laboratories.

The associate referee acknowledges the receipt of a resolution from the Nutritional Committee of the Poultry Science Association requesting in part that the present tentative method be retained as tentative for one year, or until such time as various revisions have been considered. The resolution also cites certain objections to the present method. In order to assure this group and all others interested that the basis of the method and technic is sound, it is intended to give further study to the method.

There is included in this report the results of an experiment on one of the fundamentals of the test; that is, the required length of feeding period. An experiment is also in progress to determine the effect on calcification of variations in the iodine content of the basal ration. A disturbed functioning of the thyroid may possibly affect bone development.

Vitamin D carriers also vary in their iodine content. The subject should, therefore, be investigated as a part of the study of the fundamentals of the chick assay method for antirachitic potency.

In last year's report, *This Journal*, 18, 341 (1935), two possible methods for the assay of proprietary feeds for their antirachitic potency were proposed, and the associate referee was instructed to carry out additional

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 47 (1936).



investigational work on the problem. No laboratory work was carried out in connection with these instructions. No further possible methods of approach to the problem were presented. The extraction method, in which a fat solvent is used to extract the feed, followed by a subsequent assay of the extract, failed to give complete recovery of definite additions of the antirachitic factor. Furthermore, the actual complete extraction on a routine basis of the relatively large quantities of feed necessary to give the needed quantities of extract is very difficult. The simpler method proposed last year was based on the dilution of the proprietary feed with the regular basal rachitic ration. The modified feed is then fed in accordance with the tentative method technic and its calcifying properties determined. The procedure might readily be placed on a routine basis.

Last year the associate referee stated he was of the opinion that a proprietary feed, claimed to contain a vitamin D carrier as an ingredient, should be expected to produce satisfactory calcification when diluted 25 per cent with the basal ration. Among the trade the general opinion has not been in harmony with this proposal. The view point is that the feeds need not necessarily be compounded to contain an excess of the antirachitic factor. They consider the proposal to be an injustice to the manufacturer. The trade also believes that the extraction method is not fair to them, because they can only be expected to add sufficient antirachitic factor to their own formulas to be effective under ordinary farm conditions. Even if complete extraction of the antirachitic factor could be effected, the assay method would substitute another ration and different conditions. It is to be hoped that opinion on this subject will soon crystallize and that other methods may be proposed so that a more definite objective for further study will be presented.

#### EXPERIMENTAL WORK AND DISCUSSION

Based on the findings of Lachat, Halvorson, and Palmer, *This Journal*, 15, 660 (1932), the feeding period of the method of vitamin D assay as first proposed to this association, *Ibid.*, 222, was shortened from 5 weeks to 4 weeks. A feeling persists that this four-week period is not adequate. By using the tentative method technic, Kline, Elvehjem, and Halpin<sup>1</sup> have shown that chicks from parent flocks, receiving various levels of vitamin D, rapidly and quite uniformly become severely rachitic. Even at three weeks they found a wide spread between the basal groups and the basal groups supplemented with adequate vitamin D. They conclude that the four-week period is the best length of time for the assay. It seemed desirable to obtain more data on this important subject, especially when feeding varying quantities of vitamin D.

The technic of the tentative method was again used in this experiment. The associate referee gratefully acknowledges the assistance of M. J.

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<sup>1</sup> *Poultry Sci.*, 14, 116 (1935).

Killian, L. E. Clifcorn, W. S. Thompson, Miss E. Gundlach, and J. A. Milliff. Two hundred one-day old white leghorn chicks obtained from a commercial hatchery were divided into 17 groups. The parent flock, as reported by the hatchery, received a mash feed containing 1 per cent of cod liver oil of unknown potency and scratch feed. Beginning on the third day, four groups were fed Ration 1, the basal rachitic ration supplemented with one-half of one per cent of corn oil; four groups were fed Ration 2, the same basal ration supplemented with  $\frac{1}{8}$  of one per cent of cod liver oil and  $\frac{3}{8}$  of one per cent of corn oil; four groups were fed Ration 3, the same ration supplemented with  $\frac{1}{4}$  of one per cent of cod liver oil and  $\frac{1}{4}$  of one per cent of corn oil; and four groups were fed Ration 4, the same basal ration supplemented with  $\frac{1}{2}$  of one per cent of cod liver oil. The cod liver oil was a composite of thirteen samples of poultry grade oil, containing approximately 108 U.S.P. units of vitamin D per gram. This oil had repeatedly produced complete protection against rickets by the tentative method when fed at  $\frac{1}{4}$  of one per cent. On the basis of the rat assay, Rations 2, 3, and 4 contained additions of approximately 13.5, 27, and 54 U.S.P. units of vitamin D per 100 grams of ration.

One group of the three-day old chicks was killed at the beginning of the feeding period, and the left tibia of each bird was removed for ash analysis. Each week for four consecutive weeks thereafter, four groups were killed for the same purpose, each group having received one of the four rations.

The average moisture and fat-free tibia ash analyses, together with average individual chick weights when killed, and numbers per group are given in the table.

The average tibia ash of the group killed at the outset of the feeding period was 37.3 per cent. At the end of the first week the tibia ash averages for Rations 1, 2, 3, and 4 were 40.6, 42.2, 42.8, 42.2 per cent, respectively. At the end of the second week in the same order they were 37.4, 42.7, 42.3, and 44.7 per cent. At the end of the third week in the same order they were 31.1, 42.6, 44.1, and 45.5 per cent, and at the end of the fourth week they were 31.2, 42.2, 45.7, and 47.6 per cent.

The data clearly indicate that the birds on the basal rachitic ration, Ration 1, had become as severely rachitic by the end of the third week as they were at the end of the fourth week when measured by tibia ash averages. The ash averages of the birds receiving the cod liver oil additions were practically identical at the end of the first week and were only slightly higher than those on the supplemented basal ration. The spread in tibia ash averages between the basal group and the others was increased considerably by the end of the third week. Rations 3 and 4, but not Ration 2, contained sufficient vitamin D to continue a normal increase of the calcification which is characteristic of age in birds receiving ample of the antirachitic factor. The development of rickets and the widening of

the spread in tibia ash averages between protected birds and unprotected birds, as shown in this experiment, are similar to those reported by Kline and associates.

*Effect of various levels of vitamin D intake and of the age of chicks on tibia bone ash averages*

| AGE WHEN KILLED                         | AVERAGE PER CENT ASH OF MOISTURE AND FAT-FREE TIBIAE OF GROUPS RECEIVING BASAL RATION AND COD LIVER OIL SUPPLEMENTED BASAL RATIONS, AVERAGE INDIVIDUAL CHICK WEIGHTS IN GRAMS, AND NUMBERS PER GROUP |  |  |  |
|---|--|--|--|--|
|   | RATION 1<br>BASAL  | RATION 2<br>BASAL PLUS 1% OIL<br>APPROX. 13.5 U.S.P.<br>UNITS OF VITAMIN D<br>PER 100 GRAMS OF<br>RATION | RATION 3<br>BASAL PLUS 1% OIL<br>APPROX. 27 U.S.P.<br>UNITS OF VITAMIN D<br>PER 100 GRAMS OF<br>RATION | RATION 4<br>BASAL PLUS 1% OIL<br>APPROX. 54 U.S.P.<br>UNITS OF VITAMIN D<br>PER 100 GRAMS OF<br>RATION |
| Start of feeding period<br>(3 days old) | 37.3<br>10, (35.0)   |  |  |  |
| End of first week                       | 40.6<br>11, (57.3)   | 42.2<br>11, (57.7)   | 42.8<br>12, (61.0)   | 42.2<br>12, (55.2)   |
| End of second week                      | 37.4<br>10, (81.2)   | 42.7<br>11, (87.7)   | 42.3<br>12, (81.3)   | 44.7<br>11, (89.0)   |
| End of third week                       | 31.1<br>12, (103.8)  | 42.6<br>11, (118.6)  | 44.1<br>10, (135.0)  | 45.5<br>8, (135.0)   |
| End of fourth week                      | 31.2<br>11, (107)  | 42.2<br>10, (156.5)  | 45.7<br>12, (171.7)  | 47.6<br>8, (197.5)   |

### SUMMARY

1. The four-week feeding period, as incorporated in the present tentative chick assay method for vitamin D carriers, is of sufficient duration.

2. Not more than 27 U.S.P. units of vitamin D from cod liver oil per 100 grams of basal ration are required to protect birds completely against rickets by the present tentative method when complete protection is considered to mean an average tibia ash average of more than 44 or 45 per cent.

### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That study be continued on the present tentative method for vitamin D assay by preventive biological test with a view to possible revision and collaborative study.

(2) That the investigational work on the antirachitic potency of proprietary feeds be continued.

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 47 (1936).

REPORT ON HYDROCYANIC ACID IN  
GLUCOSIDE-BEARING MATERIALS

By ROBERT A. GREENE (*Associate Referee*) and J. A. WILLIAMS  
(Arizona State Laboratory, Tucson, Ariz.)

The present tentative methods for the determination of hydrocyanic acid in glucoside-bearing materials give widely varying results in the hands of different analysts, as was shown by the report of the associate referee in 1934, *This Journal*, 17, 182 (1934). This report shows that the alkaline titration method was the least satisfactory, chiefly because of lack of a sharp end point and also because of the reduction of silver ions by volatile organic compounds. The Prussian blue method also presents difficulties, chiefly in the rate of settling of the particles of Prussian blue.

It appears that an accurate volumetric method for determining hydrocyanic acid should be preferable to a colorimetric method, since in either case the acid must be separated by hydrolysis and steam distillation. If hydrocyanic acid could be determined by a direct titration, further procedure would be eliminated.

During the past few years the writers have used a slight modification of the alkaline titration method, which has given excellent results, and in which the end point is extremely sharp and definite. The method is essentially that of Sharwood<sup>1</sup> as given by Blasdale,<sup>2</sup> and the end point depends upon the formation of a precipitate of silver iodide, rather than of silver cyanide. Because of the slight solubility of silver iodide, and the fact that in low concentrations it forms a precipitate that coagulates with difficulty, the resulting end point is very sharp. In the presence of ammonia, silver cyanide does not separate, so that the end point is the formation of silver iodide, rather than of silver cyanide.

In order to test its applicability, several samples of linseed meal were analyzed according to a modified alkaline titration method. This method has been published, *This Journal*, 19, 94 (1936).

Blasdale points out that the volume of the solution to be titrated should not exceed 100 cc., but the writers were able to secure satisfactory results with volumes as large as 160 cc. It was found that if large samples are used (20 grams or more) all the hydrocyanic acid may not be carried over in 150 cc. of distillate. In these cases it is advisable to distil a larger volume, then acidify the distillate with dilute sulfuric acid and re-distil with steam. This will give large enough amounts of hydrocyanic acid for a satisfactory titration.

The following table gives the results of the analysis of several samples of linseed meal by a number of analysts. For the sake of comparison, titrations were also made by the alkaline titration method.

<sup>1</sup> *J. Am. Chem. Soc.*, 19, 400 (1897).

<sup>2</sup> *The Fundamentals of Quantitative Analysis*, D. Van Nostrand Co., pp. 289-296 (1928).

TABLE 1.—*Collaborative results, hydrocyanic acid*

| ANALYST         | SAMPLE NO. | ALKALINE TITRATION METHOD |        | MODIFIED ALKALINE TITRATION METHOD |        | COLORIMETRIC METHOD |       |
|-----------------|------------|---------------------------|--------|------------------------------------|--------|---------------------|-------|
|                 |            | p.p.m.                    |        | p.p.m.                             |        |                     |       |
| R. A. Greene    | I          | 343.9                     |        | 304.8                              |        |                     |       |
|                 |            | 333.8                     | 335.1  | 300.9                              | 301.46 |                     |       |
|                 |            | 327.6                     |        | 298.7                              |        |                     |       |
| J. A. Williams  | I          | 311.4                     |        | 300.9                              |        |                     |       |
|                 |            | 327.6                     | 321.8  | 304.8                              |        |                     |       |
|                 |            | 331.4                     |        | 304.8                              | 304.02 |                     |       |
|                 |            | —                         |        | 304.8                              |        |                     |       |
|                 |            | —                         |        | 304.8                              |        |                     |       |
| R. A. Greene    | II         |                           |        | 190.5                              |        |                     |       |
|                 |            |                           |        |                                    | 190.5  |                     |       |
| J. A. Williams  | II         |                           |        | 190.5                              |        |                     |       |
| R. A. Greene    | III        | 385.8                     |        | 206.55                             |        | 150.0               |       |
|                 |            | 440.6                     | 394.8  | 206.55                             |        | 153.0               |       |
|                 |            | 358.0                     |        | 206.55                             | 199.38 | 122.4               | 138.2 |
|                 |            |                           |        | 192.78                             |        | 122.4               |       |
|                 |            |                           |        | 184.5                              |        | 134.6               |       |
|                 |            |                           |        |                                    |        | 146.8               |       |
| J. A. Williams  |            | 371.5                     |        | 192.78                             |        | 122.4               |       |
|                 |            |                           |        | 206.55                             |        | 134.6               |       |
|                 |            |                           |        | 158.3                              |        | 122.4               |       |
|                 |            |                           |        | 155.3                              | 168.95 | 138.7               | 135.7 |
|                 |            |                           |        | 154.2                              |        | 146.8               |       |
|                 |            |                           |        | 166.24                             |        | 153.0               |       |
|                 |            |                           |        | 154.12                             |        | 133.9               |       |
|                 |            |                           |        | 154.12                             |        | 133.9               |       |
|                 |            |                           |        | 179.0                              |        |                     |       |
| J. F. Breazeale | III        | 399.33                    |        | 192.78                             |        |                     |       |
|                 |            | 358.11                    | 378.72 | 192.78                             | 192.78 |                     |       |
| E. L. Breazeale | III        | 495.72                    |        | 151.36                             |        |                     |       |
|                 |            | 330.49                    | 413.1  | 192.78                             | 172.07 |                     |       |
| H. V. Smith     | III        | 444.2                     |        | 165.13                             |        |                     |       |
|                 |            |                           |        | 165.13                             | 162.41 |                     |       |
|                 |            |                           |        | 156.98                             |        |                     |       |
| Average, Sample | III        | 398.6                     |        | 177.84                             |        | 136.9               |       |

In Table 1 it is seen that the several analysts secured fair agreement when titrations were made in the presence of ammonia and potassium iodide, but that their results by the alkaline titration method vary

widely. The lack of agreement in the former case is partly due to the fact that owing to the small quantity of hydrocyanic acid in the aliquot titrated, only a small amount of silver nitrate was required. Consequently, in expressing results in terms of parts per million, slight discrepancies become significant due to multiplication. There is no great difficulty in recognizing the end point, and apparently the only precaution to be taken is to add the silver nitrate slowly and with constant stirring.

#### COLORIMETRIC METHODS

The principal advantage of the colorimetric methods is the elimination of the indefinite end point of the alkaline titration method. However, considerable additional time is required for the determination. The Prussian blue method has been used for several years, and while it has apparently given satisfactory results in the hands of various analysts, there are some difficulties involved. The fact that some of the operations must be made under diminished pressure is, in the opinion of the writers, an objectionable feature. In addition, some analysts do not care to use a standard solution of potassium cyanide. Due to a lack of time for investigation, the writers are not prepared to present any further comments at present regarding this method.

Another colorimetric method is that of Francis and Connell.<sup>1</sup> Several determinations were made by this method, and fairly satisfactory results were obtained. The original method was slightly modified as follows:

Hydrolysis and distillation are carried out according to the official methods.<sup>2</sup> The distillate is collected in dilute sodium hydroxide, the distillate is made to a convenient volume, usually 250 cc., and an aliquot of 50–100 cc. is placed in a beaker or evaporating dish; 1 cc. of yellow ammonium sulfide is added, and the solution is evaporated to dryness on a steam bath. The residue is taken up in 15 cc. of hot water, and the solution is filtered through a small quantitative filter paper. The filtrate is acidified with dilute (approximately 0.5 *N* HCl) and is boiled for 5 minutes. The solution is filtered, and if not clear, 1 cc. of the dilute acid is added, and the solution is boiled again and filtered. Several similar treatments with acid may be necessary to secure a water-clear filtrate, but the authors have found that usually two such treatments are sufficient, and that a slight turbidity does not greatly interfere in the subsequent color comparisons.

The solution is then transferred to a Nessler tube or a small volumetric flask. It is advisable to keep the volume under 50 cc. unless a large sample has been used or it is known that the aliquot used contains relatively large amounts of hydrocyanic acid.

The standard is prepared as follows: 19 grams of potassium thiocyanate (KSCN) is dissolved in 1000 cc. of water and standardized gravimetri-

<sup>1</sup> *J. Am. Chem. Soc.*, 35, 1624 (1913).

<sup>2</sup> *Methods of Analysis*, A.O.A.C., 1930.

cally with silver, and the solution is adjusted so that 1 cc. contains 14.92 mg. of potassium thiocyanate, which is equal to 10 mg. of potassium cyanide. Since it is preferable to report results in terms of hydrocyanic acid, the above standard is somewhat awkward to use. Consequently, the authors recommend the following modification of the procedure as given by Francis and Connell:<sup>1</sup>

Dissolve approximately 36.0 grams of KSCN in a liter of distilled water and standardize volumetrically by titrating with standard  $\text{AgNO}_3$  solution, using ferric ammonium sulfate as an indicator. Adjust the solution so that 1 cc. contains 35.9632 mg. of KSCN; 1 cc. of this solution is equivalent to 10 mg. of HCN, and by dilution of this solution a standard of any desired strength may be obtained. (The authors recommend that 10 cc. of stock KSCN solution be diluted to a liter; 10 cc. of this diluted solution will be equivalent to 0.1 mg. of HCN.)

Make up a series of standards by placing various amounts of the dilute standard solution in small volumetric flasks or Nessler tubes. Now add 15 drops of 5% ferric chloride solution to the standards and the unknowns, which are diluted to volume, mixed and compared in a colorimeter. (The Kennicott-Sargent or the Kennicott-Campbell-Hurley type gives satisfactory results.)

In comparing the colors of the standard and the unknown samples, use the standard nearest to the color of the unknown. If a Kennicott type colorimeter is used, rather than the conventional biological type, use a standard of such strength that if the tube containing the unknown solution has a volume of 50 cc., the reading of the standard is in the range of 20–30 cc. Therefore, if the unknown solution contains 0.2 mg. of HCN in a volume of 50 cc., 100 cc. would be equivalent to 0.8–1.0 mg. of HCN.

The authors have found this method to be superior to the Prussian blue method. In the first place, it is more sensitive, since equal quantities of standard give a more intense color of ferric sulfocyanate (particularly in lower concentrations) than the color of Prussian blue. In addition, the conditions for developing the color of ferric sulfocyanate are not so rigorous as those for the development of Prussian blue. This method also eliminates distillation under reduced pressure, with subsequent transfer of the material, and the possibilities of loss due to spattering during the distillation or the transfer.

#### PHOTO-ELECTRIC METHODS

Within recent years photo-electric methods have been introduced as a means of eliminating personal errors in colorimetric or turbidimetric work. Bartholomew and Raby<sup>2</sup> have used such a method with success, and therefore a sample of the linseed meal was sent to them. The determination of hydrocyanic acid was carried out in about the same manner as indicated above, except that a one liter Wellman flask was used instead of a Kjeldahl flask. The titrations were made by Sharwood's<sup>3</sup> method, and the use of the photo-electric set-up previously described by the

<sup>1</sup> *Loc. cit.*

<sup>2</sup> *Ind. Eng. Chem. Anal. Ed.*, 7, 68 (1935).

<sup>3</sup> *J. Am. Chem. Soc.*, 19, 400 (1897).

writers. The results are given in Table 2. In each case the results agree very well, and they also agree fairly well with those obtained colorimetrically. It appears that this method gives the most accurate results of any of the methods employed, and is to be preferred when a photo-electric apparatus is available.

TABLE 2.—*Results of tests for autogenous hydrocyanic acid in flaxseed meal*  
(Analyses made by E. T. Bartholomew and E. C. Raby, University of California, Riverside, 1935. The meal was furnished by the associate referee)

| TEST NO. | SAMPLE, FLAX MEAL | MACERATING TEMPERATURE | VOL. OF DISTILLATE | DISTILLATE MADE UP TO— | VOL. OF ALIQUOT TITRATED | AgNO <sub>3</sub> TITRATION VALUES | TOTAL HCN IN SAMPLE |
|----------|-------------------|------------------------|--------------------|------------------------|--------------------------|------------------------------------|---------------------|
|          | grams             | °C.                    | cc.                | cc.                    | cc.                      | cc.                                | mg.                 |
| 1        | 10                | 24.5                   | 150                | 500                    | 150                      | .78, .78                           | 1.40                |
| 2        | 10                | 20.0–21.0              | 150                | 500                    | 150                      | .78, .78                           | 1.40                |
| 3        | 10                | 24.0–25.0              | 150                | 500                    | 150                      | .77, .77                           | 1.38                |
| 4        | 10                | 20.0–21.0              | 150                | 1000                   | 150                      | .41, .41                           | 1.48                |
| 5        | 20                | 22.5–23.0              | 150                | 500                    | 150                      | 1.56, 1.56, 1.56                   | 2.80                |

#### CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS

There is no provision, at present, for qualitative tests for determining the presence of cyanogenetic glucosides in plants, feeds, and similar materials. During the past few years, the writers have used a test suggested by Guignard,<sup>1</sup> and published by Morrow.<sup>2</sup> The method has also been published in *This Journal*, 19, 94 (1936).

Another qualitative test, which was not tried, is that of Fox,<sup>3</sup> in which the hydrocyanic acid is aerated into a potassium iodide solution. Fox claims that the method has an accuracy of about 1 part in 2,000,000 and is also more specific than the Guignard test.

#### DISCUSSION

Sharwood's method gives a much more definite end point than does the old alkaline titration method, but in spite of the sharpness of this end point there is some individual variation, as shown in Table 1. However, under carefully controlled conditions the method should give satisfactory results. The colorimetric method of Francis and Connell also shows promise. The photo-electric method is probably the most accurate of all. Other methods, which were not tried but which have been recommended, are those of Roe<sup>4</sup> and Bishop.<sup>5</sup>

Bartholomew and Raby<sup>6</sup> pointed out that there is the possibility of loss of hydrocyanic acid during the process of autolysis if it is not carried

<sup>1</sup> *Bull. Sci. Pharmacol.*, 13, 129–138, 193–213, 337–352, 401–419, 415–417 (1906).

<sup>2</sup> *Biochemical Laboratory Methods*, John Wiley & Sons, New York (1927).

<sup>3</sup> *Science*, 79, 37 (1934).

<sup>4</sup> *J. Biol. Chem.*, 58, 667 (1924).

<sup>5</sup> *Biochem. J.*, 21, 1162 (1927).

<sup>6</sup> *Loc. cit.*



out in a closed container. They suggest that the distillation apparatus be connected and that sufficient water be added so that it will not be necessary to disassemble the apparatus, thereby reducing the possibility of loss of hydrocyanic acid.

#### RECOMMENDATIONS<sup>1</sup>

It is recommended—

(1) That the autolysis be conducted with the apparatus completely connected and sufficient water be added initially so it will be unnecessary to disconnect the apparatus until distillation is complete.

(2) That the modified alkaline titration method (using ammonia and KI) be tentatively adopted.

(3) That the photo-electric method be adopted.

(4) That further study be given to the Prussian blue method and the colorimetric method of Francis and Connell.

(5) That qualitative tests for the detection of cyanogenetic glucosides be adopted.

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#### REPORT ON FAT IN DAIRY PRODUCTS USED AS FEEDS

By W. CATESBY JONES (Department of Agriculture,  
Richmond, Va.), *Associate Referee*

Collaborative work was undertaken in 1933 under the direction of the associate referee. This work was quite satisfactory, but it was not extensive enough to warrant any definite conclusions. The work was continued under the present referee in 1934. Here again the results failed to justify any conclusions as to the most suitable of the three methods studied, namely, the Roese-Gottlieb, the Roese-Gottlieb Modified, and the Acid Hydrolysis. However, since the acid hydrolysis method gave slightly higher results than the Roese-Gottlieb methods, it was decided to study a modified acid hydrolysis method this year. This method differs from the one previously studied in that the fat is extracted from a solid phase instead of a liquid phase. The method is similar to the one used for the determination of fat in bread.

The associate referee obtained a large sample of dried buttermilk and sent samples to twelve collaborators with the request that the fat content be determined by the modified acid hydrolysis method on a moisture-free basis.

Owing to other duties, four of the twelve collaborators were unable to assist in this work. The table will give an idea of the variance between collaborative results by this method. The extreme spread between high and low is 2.07 per cent. The average for all analyses is 4.86 per cent,

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 47 (1936).

with a plus difference from the average of 1.06 per cent and a minus difference of 1.01 per cent.

The following is a summary of the comments given by the collaborators: (1) The method is too long and cumbersome; (2) it is tedious; (3) it gives no greater yield in fat; (4) it requires large quantities of reagents; and (5) it is very difficult to get uniform checks with duplicate samples.

It is recommended<sup>1</sup> that further work on the modified hydrolysis method in connection with dairy products be discontinued. It is suggested that the associate referee cooperate with an Associate Referee on Solvents for the Determination of Fat in Feeding Stuffs, in studying the acid hydrolysis method for the determination of fat in stock feeds. This is in compliance with recommendations made by the Committee on Recommendations of Referees. It is further suggested that this work be done on samples received by the various control laboratories and not on a collaborative basis.

*Collaborative results on fat in dairy products used as feeds*

**DRIED BUTTERMILK**

| Collaborators      | Hillig Hydrolysis<br>per cent |
|--------------------|-------------------------------|
| 1                  | 5.92                          |
| 2                  | 4.55*                         |
| 3                  | 5.35*                         |
| 4                  | 4.73                          |
| 5                  | 4.95                          |
| 6                  | 4.30*                         |
| 7                  | 12.93†                        |
| 8                  | 3.85                          |
| 9                  | 5.23*                         |
| Average            | 4.86                          |
| Extreme Difference | 2.07                          |
| + Diff. from Av.   | 1.06                          |
| - Diff. from Av.   | 1.01                          |

\* Checked by the Rose-Gottlieb tentative method with an average result of 5.03 %

† Result not included in average.

## REPORT ON BIOLOGICAL METHODS FOR VITAMIN B COMPLEXES

By C. A. ELVEHJEM (Department of Agricultural Chemistry,  
University of Wisconsin, Madison, Wis.),  
*Associate Referee*

In a previous report, *This Journal*, 18, 354 (1935), a method was proposed for the determination of vitamin B (B<sub>1</sub>) or the antineuritic vitamin

<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936).

in feeding stuffs. The method consists essentially of feeding day-old chicks Ration 242A and determining what percentage of the material to be assayed is necessary as a supplement to the basal ration in order that all the birds in the group are protected completely against polyneuritis.

Ration 242A has the following composition:

| <i>Autoclaved portion:</i>                                | <i>per cent</i> |
|---|-----------------|
| Ground yellow corn.....                                   | 57              |
| Pure flour middlings.....                                 | 25              |
| Crude domestic acid precipitated casein.....              | 12              |
| <i>Untreated portion:</i>                                 |                 |
| Vacuum desiccated whole liver substance <sup>1</sup> .... | 2               |
| Calcium carbonate (precipitated).....                     | 1               |
| Calcium phosphate (precipitated).....                     | 1               |
| Iodized salt (0.02% potassium iodide).....                | 1               |
| Cod liver oil.....  | 1               |

During the past year this method has been used rather extensively in the associate referee's laboratory for the determination of vitamin B in biological materials. In each case the smallest level of material necessary to protect all the chicks in the group over a period of 5 weeks was determined. Any material fed on the percentage basis that protected the chicks for five weeks was found to continue to do so indefinitely. Excellent results were obtained in the case of a large number of animal tissues. The animals receiving a level of vitamin B sufficient for protection against polyneuritis showed good growth, and clear-cut differences between rather small increments in the material assayed were obtained. However, the results with certain samples of yeast were not so clear cut. The level of yeast necessary to give complete protection against polyneuritis could be determined with a fair degree of accuracy, but the growth of the chicks was somewhat retarded when yeast, and especially crystalline vitamin B, was used as the source of the antineuritic factor. This, it is believed, is due to certain variations in the amount of the other components of the vitamin B complex in the basal ration and in the chicks at birth. The same sample of yeast when assayed at different times of the year will give the same degree of protection against polyneuritis, but the rate of growth to the protected birds will vary to a considerable extent. This difficulty is being remedied by adding certain materials to the basal ration to supply the additional factors without increasing the vitamin B content. Until the details of this modification are completed the proposed method cannot be recommended as a tentative method.

#### EXPERIMENTAL

Several additional assays have been conducted on the standard yeasts, No. 1 and No. 2, which were used in the experimental work reported last year. Another yeast, standard yeast No. 3, obtained from E. M. Nelson,

<sup>1</sup> Wilson Laboratories, Chicago.

TABLE 1.—*Vitamin B assay of standard yeasts I, II, III, four chicks*

| GROUP<br>NUMBER | SUPPLEMENT      | CHICKS<br>SURVIVING<br>5 WEEKS | WEIGHT<br>AT<br>5 WEEKS | TIME OF<br>POLYNEURITIS |
|-----------------|-----------------|--------------------------------|-------------------------|-------------------------|
|                 | <i>per cent</i> |                                | <i>grams</i>            | <i>days</i>             |
| 123             | Basal           | None                           | —                       | 9-11                    |
| 113             | 1.5, yeast I    | 3                              | —                       | 17                      |
| 114             | 1.75 "          | 4                              | 145                     | None                    |
| 115             | 2.0 "           | 4                              | 162                     | "                       |
| 116             | 2.25 "          | 4                              | 159                     | "                       |
| 6               | Basal           | None                           | —                       | 10                      |
| 12              | 1.0, yeast II   | "                              | —                       | 10-14                   |
| 77              | 1.25 "          | "                              | —                       | 18-27                   |
| 13              | 1.5 "           | 4                              | 157                     | None                    |
| 14              | 2.0 "           | 4                              | 235                     | "                       |
| 15              | 2.5 "           | 4                              | 183                     | "                       |
| 33              | Basal           | None                           | —                       | 10                      |
| 38              | 1.5, yeast III  | "                              | —                       | 10-14                   |
| 39              | 1.75 "          | "                              | —                       | 14-21                   |
| 40              | 2.0 "           | 4                              | 110                     | None                    |
| 127             | 2.25 "          | 4                              | 137                     | "                       |

Washington, D. C., was also tested. This yeast contains 10 international units of vitamin B per gram. Typical results are given in Table 1. It is evident that the chicks respond very differently to levels of yeast differing by only 0.25 per cent. In the case of yeast No. 1, the 1.5 per cent level did not give complete protection, but 1.75 per cent prevented polyneuritis in all the birds and produced good growth. Thus, the protective level is established at 1.75 per cent. Last year the protective level was set at 2 per cent, but levels intermediate between 1.5 and 2.0 per cent were not fed. It appears impractical to feed levels differing by less than 0.25 per cent.

For yeast No. 2 the 1.25 per cent level did not give protection, but 1.5 per cent protected all the birds from polyneuritis. There is some variation in the growth records, but the growth cannot be considered poor in any case. The protective level is therefore established at 1.5 per cent.

Yeast No. 3 did not give complete protection until 2 per cent was fed. The growth in this case was rather poor, but improved growth has been obtained on modified rations. Since this yeast contains 10 international units per gram, the potency of the other yeasts in terms of international units can be calculated. However, it is better to express the potency on the percentage basis until a more complete comparison has been made.

#### COLLABORATIVE STUDY

Samples of standard yeasts No. 1 and No. 2 were assayed by F. L. Gunderson, Quaker Oats Company, Chicago. The results obtained in his

laboratory are given in Table 2. The work in the two laboratories checks very well. He also found that 1.75 per cent of yeast No. 1 and 1.5 per cent of yeast No. 2 were necessary to give complete protection. There were some slight differences in the rates of growth, but they were not sufficient to be significant. In the case of yeast No. 1, Gunderson continued

TABLE 2.—*Vitamin B assay of standard yeasts I and II*  
(Results obtained by Gunderson)

| GROUP<br>NUMBER | SUPPLEMENT      | NUMBER<br>CHICKS | CHICKS<br>SURVIVING<br>AT 6 WEEKS | WEIGHT<br>AT<br>6 WEEKS | TIME OF<br>POLYNEURITIS |
|-----------------|-----------------|------------------|-----------------------------------|-------------------------|-------------------------|
|                 | <i>per cent</i> |                  |                                   |                         | <i>days</i>             |
| 124             | Basal           | 12               | None                              | —                       | 10-13                   |
| 128             | 1.5, yeast I    | 5                | 1                                 | —                       | 14-27                   |
| 129             | 1.75 "          | 5                | 5                                 | 255                     | None                    |
| 130             | 2.0 "           | 5                | 5                                 | 301                     | "                       |
| 131             | 4.0 "           | 5                | 5                                 | 326                     | "                       |
|                 |                 |                  | Chicks<br>surviving<br>at 4 weeks | Weight at<br>4 weeks    |                         |
| 132             | Basal           | 10               | None                              | —                       | 5-10                    |
| 140             | 1.5, yeast II   | 10               | 10                                | 193                     | None                    |
| 141             | 1.62 "          | 10               | 9                                 | 190                     | "                       |
| 142             | 1.75 "          | 10               | 8                                 | 192                     | "                       |
| 143             | 4.0 "           | 10               | 10                                | 183                     | "                       |

the tests for 6 weeks and when yeast No. 2 was tested the experiments were discontinued at 4 weeks. There is still some question regarding the time. A 5-week period is ample, and it may be possible to reduce the time to 4 weeks.

The preliminary collaborative work indicates that this method gives results that can be duplicated by competent workers in any laboratory.

It is recommended that studies and collaborative work on the proposed method be continued.

## REPORT ON TECHNIC AND DETAILS OF BIOLOGICAL METHODS, VITAMIN D CARRIERS

By LAWRENCE LYSLE LACHAT (Feed Laboratory, Department  
of Agriculture, Dairy and Food, St. Paul, Minn.),  
*Associate Referee*

The purpose of this report is to present comparative studies of bone ash methods used in this and other collaborating laboratories. Investiga-

tions started by the associate referee last year, *This Journal*, 18, 357 (1935), were continued for it seemed urgently desirable to study and evaluate fundamental factors influencing the practical applicability of vitamin D assays. At present the interpretation of chick feeding tests is largely based on bone ash criteria, and failure to standardize the determination employed may seriously affect the degree of accuracy desired, especially when assays are conducted in other laboratories by different technicians.

Recently, methods of dissection and preparation of bone for analysis have been studied by St. John, Kempf and Bond,<sup>1</sup> Bethke and Record,<sup>2</sup> and Harshaw, Fritz and Titus.<sup>3</sup> Experiments in the associate referee's laboratory are in progress concerning this important phase of the problem. Also being continued are studies involving the use of different lipid solvents for extraction purposes. Unfortunately, at this time these investigations do not include a sufficient number of samples to warrant final comparisons in this report.

During the preceding years, the work of promoting greater uniformity and accuracy in analysis of bone by collaboration with biological feeding laboratories has been continued. Comparative summaries obtained by this means, when calculated by statistical methods, show differences that are common in ash determinations, especially when methods differing from the one employed by the associate referee are used. Further collaborative material has been assembled to compare results when a standardized method is employed in different laboratories on identical material. These studies have offered additional support for the belief of the associate referee that concordant results can be obtained among various laboratories using the same sample, providing the analytical procedures are alike and details followed empirically.

For convenience the experiments in this report have been separated into two general divisions to compare results when samples are analyzed by various laboratories using different methods with those obtained when the samples are analyzed by the associate referee's method.

#### RESULTS OBTAINED BY USING DIFFERENT METHODS

Several biological feeding laboratories kindly furnished a series (samples A) of chick tibiae dissected and freed of tissue exhibiting various degrees of calcification. The opposite tibiae (samples B) were also dissected and freed of tissue in exactly the same manner. Bone ash determinations of samples B were then made by these laboratories without regard to the method employed by the associate referee. The results of analysis were then compared with similar results obtained from samples A analyzed by the associate referee's method.

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<sup>1</sup> *Poultry Sci.*, 12, 34 (1933).

<sup>2</sup> *Ibid.*, 13, 29 (1934).

<sup>3</sup> *J. Agr. Research*, 48, 997 (1934).

TABLE 1.—Statistical summary of comparative bone ash contents determined by various laboratories

| CHEMIST SUBMITTING<br>BONE SAMPLES | AVERAGE<br>ASH CONTENT<br>(SAMPLES B)<br><br>per cent | ANALYST ANALYZING<br>FOR ASH CONTENT<br>USING OPPOSITE TIBIAL | AVERAGE<br>ASH CONTENT<br>(SAMPLES A)<br><br>per cent | AVERAGE<br>DIFFERENCE          | P   | SIGNIFICANCE OF<br>DIFFERENCE                | REMARKS ON<br>CALCULATION        |
|------------------------------------|---|---|---|--------------------------------|---|--|----------------------------------|
|                                    |   |   |   |                                |   |  |                                  |
| F. D. Baird                        | 34.5<br>43.0  | L. S. Walker  | 37.2<br>45.4  | 2.7<br>2.4                     | less than 0.01<br>less than 0.01                          | positive<br>positive                         | poor<br>normal                   |
| L. E. Bopst                        | 31.9<br>46.9  | H. R. Kraybill  | 32.6<br>46.8  | 0.7<br>-0.1                    | less than 0.01<br>0.4 -0.3                                | positive<br>negative                         | very poor<br>normal              |
| T. A. Faust                        | 45.72*<br>45.63*<br>46.37*<br>32.98*                  | L. L. Lachat  | 52.12<br>51.61<br>52.14<br>36.39                      | 6.40<br>5.98<br>5.77<br>3.41   |   |  |                                  |
| W. B. Griem                        | 31.7<br>49.1  | G. H. Marsh   | 32.4<br>48.4  | 0.7<br>-0.7                    | 0.02-0.01<br>0.1 -0.05                                    | positive<br>negative                         | very poor<br>normal              |
| J. E. Hunter                       | 39.42<br>50.46  | L. L. Lachat  | 36.77<br>47.56  | -2.65<br>-2.90                 | less than 0.01<br>less than 0.01                          | positive<br>positive                         | poor<br>normal                   |
| W. M. Insko, Jr.                   | 35.11†<br>42.75†<br>35.09†<br>46.40†                  | L. L. Lachat  | 35.67†<br>44.41†<br>34.95†<br>45.10†                  | 0.56<br>1.66<br>-0.14<br>-1.30 | 0.1 -0.05<br>less than 0.01<br>0.6 -0.5<br>less than 0.01 | negative<br>positive<br>negative<br>positive | poor<br>normal<br>poor<br>normal |
| A. H. Mendonca                     | 36.6<br>46.6  | J. J. Vollertsen  | 37.1<br>45.4  | 0.5<br>-1.2                    | 0.3 -0.2<br>less than 0.01                                | negative<br>positive                         | poor<br>normal                   |
| A. Richardson                      | 38.08<br>46.86  | E. M. Bailey  | 39.15<br>47.10  | 1.07<br>0.24                   | 0.02-0.01<br>0.2 -0.1                                     | positive<br>negative                         | poor<br>normal                   |

\* Bone ash obtained from pooled series of four or more tibiae plus femura.

† Average ash content of femura.

The essential data are recorded in Table 1. When a difference occurred that was statistically significant, it is shown as "positive." If insignificant statistically, the difference is shown as "negative." Each collaborator included both poorly calcified and well calcified bones in two or four series of determinations. It will be noted that results mentioned in the third and fourth columns of the table were produced by other collaborators following the associate referee's method of analysis described in last year's report, while results shown in the second column were recorded on oppositely identical samples following chiefly dissimilar methods of procedure. The number comprising each series treated by statistical methods<sup>1</sup> consisted of averages from 8 to 15 tibiae.

In nearly every case the statistical significance of the differences between both poorly calcified and well calcified samples was positive. From this treatment of the data it is clearly apparent that collaborators analyzing samples B were unable to reproduce satisfactorily results produced by the associate referee's method on oppositely identical samples.

The inference to be drawn from these data is obvious—unless the methods for bone ash determinations are standardized and the procedure carefully followed in detail, results will differ. Sufficient evidence has been accumulated in the associate referee's laboratory to show conclusively that the ash content of the right and left tibiae is identical, providing abnormalities in structure and calcification are absent. It seems unlikely, in view of the careful selection made of bone samples by the collaborators, that abnormalities can account for the differences noted, but rather that the variations are due to and should be expected from differences in analytical procedures.

TABLE 2.—Statistical summary of comparative ash contents determined by a standard method

| COLLABORATOR*                     | ANALYST            | NO.<br>SAMPLES | AVERAGE ASH CONTENT |                   |                 | †                   | REMARKS                        |
|-----------------------------------|--------------------|----------------|---------------------|-------------------|-----------------|---------------------|--------------------------------|
|                                   |                    |                | COLLABO-<br>RATOR   | ASSOC.<br>REFEREE | DIFFER-<br>ENCE |                     |                                |
|                                   |                    |                | <i>per cent</i>     | <i>per cent</i>   | <i>per cent</i> |                     |                                |
| J. H. Mitchell<br>Clemson College | D. B. Roderick     | 8              | 39.47               | 39.05             | -0.42           | 0.1-0.05            | Bones not well<br>calcified    |
| R. C. Newton<br>Chicago           | H. E. Robinson     | 17             | 29.88               | 30.06             | 0.18            | 0.3-0.2             | Bones very poorly<br>calcified |
| J. J. Taylor<br>Tallahassee       | E. P. Greene       | 10             | 32.72               | 32.96             | 0.24            | 0.1-0.05            | Bones very poorly<br>calcified |
| G. S. Fraps<br>College Station    | A. R. Kemmerer     | 12             | 46.91               | 46.85             | -0.06           | greater than<br>0.9 | Bones well calci-<br>fied      |
| R. B. Dustman<br>Morgantown       | C. E. Weakley, Jr. | 11             | 46.93               | 46.97             | 0.04            | 0.7-0.6             | Bones well calci-<br>fied      |

\* Shown in the order that results were received.

† Significance of difference, negative.

<sup>1</sup> Fisher's Statistical Methods for Research Workers. Oliver and Boyd, Edinburgh, Scotland.



## RESULTS OBTAINED BY USING A STANDARDIZED METHOD

Table 2 presents additional results of collaborative experiments relating to corresponding opposite tibiae, when analyzed separately by identical methods in different laboratories. The procedure was essentially that described previously. It will be observed that the differences are small and statistically of no significance.

From a summary of the results given by the several experimental studies, it may be concluded that comparable results on identical samples of bone are obtained *only* when a standardized uniform analytical procedure is used.

RECOMMENDATIONS<sup>1</sup>

It is recommended—

- (1) That the study of various factors affecting bone ash determinations be continued.
- (2) That the study of dissection and preparation of the bone sample for analysis be undertaken.
- (3) That extraction procedures using different lipid solvents be critically evaluated.

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REPORT ON MECHANICAL CLASSIFICATION  
OF ALFALFA PRODUCTS

By W. L. HALL (Bureau of Agricultural Economics,  
Washington, D. C.), *Associate Referee*

In September of this year an open conference was called at St. Louis, Mo., and all persons or organizations interested in or concerned with the production, use or control of alfalfa meal were invited to attend and participate in the discussion. The purpose of the conference was to present to those interested the recent results of the studies of this Bureau upon ground alfalfa and also to receive criticism and suggestions pertaining to the final compilation of Official U. S. Standards for ground alfalfa.

A system of classification for alfalfa meal, based upon the percentage of leaf particles entering into the various types of meal proposed, was presented. This percentage leaf particle system was founded upon the average protein and fiber analyses for separated alfalfa leaves and stems (Table 1). The average was computed from analytical data collected from practically all the literature pertaining to alfalfa and its production in the United States in conjunction with the analyses made by the Bureau of Agricultural Economics. Only those data were chosen and used that seemed reliable and gave specific citation to analyses of separated leaves and stems, and where a choice was possible, data were selected for early

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<sup>1</sup> For report of Subcommittee A and action of the Association, see *This Journal*, 19, 48 (1936).

TABLE 1.—*Maximum, minimum, and average protein and fiber analyses of alfalfa leaves and stems*

|         | LEAVES   |          | STEMS    |          |
|---------|----------|----------|----------|----------|
|         | PROTEIN  | FIBER    | PROTEIN  | FIBER    |
|         | per cent | per cent | per cent | per cent |
| Maximum | 29.6     | 18.6     | 12.8     | 46.7     |
| Minimum | 19.9     | 11.3     | 8.2      | 33.1     |
| Average | 24.0     | 14.4     | 10.6     | 38.3     |

TABLE 2.—*Comparison of protein and fiber contents for various percentage mixtures of leaves and stems that may be considered for the subclasses of ground alfalfa*

| TYPES OF MEAL OR<br>SUBCLASSES | ASSIGNED<br>PROTEIN | CHANCES IN 100<br>OF OBTAINING<br>SUCH A MEAL<br>HAVING THE<br>ASSIGNED OR<br>HIGHER PER-<br>CENTAGE PROTEIN | ASSIGNED<br>FIBER | CHANCES IN 100<br>OF OBTAINING<br>SUCH A MEAL<br>HAVING THE<br>ASSIGNED OR<br>LOWER PER-<br>CENTAGE FIBER |
|--------------------------------|---------------------|--|-------------------|---|
|                                |                     |  |                   |   |
|                                | per cent            |  | per cent          |   |
| <b>LEAF MEAL</b>               |                     |  |                   |   |
| Containing 85% leaves          | 18.0                | 96   | 22.0              | 96  |
| 15% stems, average pro-        | 19.0                | 91   | 21.0              | 89  |
| tein calculated 22.0%,         | 20.0                | 81   | 20.0              | 82  |
| average fiber calculated       | 21.0                | 67   | 19.0              | 68  |
| 17.9%                          |                     |  | 18.0              | 51  |
| <b>SPECIAL ALFALFA MEAL</b>    |                     |  |                   |   |
| Containing 60% leaves          | 15.0                | 96   | 30.0              | 99  |
| 40% stems, average pro-        | 16.0                | 91   | 29.0              | 97  |
| tein calculated 18.6%,         | 17.0                | 80   | 28.0              | 94  |
| average fiber calculated       | 18.0                | 63   | 27.0              | 88  |
| 24.0%                          |                     |  | 26.0              | 76  |
| <b>ALFALFA MEAL</b>            |                     |  |                   |   |
| Containing 25% leaves          | 12.0                | 89   | 36.0              | 89  |
| 75% stems, average pro-        | 13.0                | 72   | 35.0              | 82  |
| tein calculated 14.0%,         | 13.5                | 61   | 34.0              | 71  |
| average fiber calculated       |                     |  | 33.0              | 59  |
| 32.3%                          |                     |  |                   |   |
| <b>STEM MEAL</b>               |                     |  |                   |   |
| Containing less than           | —                   | —  | —                 | —   |
| 25% leaves                     |                     |  |                   |   |

cut alfalfa. Variations in moisture were corrected by recalculating the data to the average moisture found by this laboratory for air-dried samples. From these data and certain selected leaf-stem ratios the probability or chance of obtaining a corresponding meal having assigned protein and fiber values for the particular leaf-stem ratio was calculated by the usual statistical methods (Table 2). Essentially the idea behind the whole scheme is that it is this ratio of leaves to stems that determines the protein-fiber value range of a meal. Those attending the conference concluded that the leaf percentage system of classification possessed certain difficulties because of impracticability and that the double standard, namely protein and fiber, should be incorporated in the Official Standards for alfalfa. The point was emphasized that essentially there is such a double standard today, and the necessary protein-fiber analyses are made for the commerce of alfalfa meals.

It is planned, therefore, to issue Official U. S. Standards for ground alfalfa, and to use fiber and protein as the chemical classifying factors for the differentiation of the subclasses, namely, alfalfa leaf meal, special alfalfa meal, alfalfa meal, and alfalfa stem meal. Even under this scheme there will be cases, perhaps few in number, where a particular meal will be sufficient in the chemical factors, protein and fiber, and yet to the trained eye be lacking in the expected percentage of leaf particles. Whether such a meal would meet the nutritional needs for which it was intended is problematical. An inspection service would be of value in this connection, but because of lack of funds such a service cannot be undertaken at this time. There are certain commercial concerns, such as the mixed feed manufacturers and some alfalfa millers, who might welcome an inspection service. Accompanying the standards would be published some of the results of research upon alfalfa meal.

No report on qualitative tests for proteins was given by the associate referee.

#### CORRECTIONS

In *This Journal*, 19, 94 (1936), line 6, change "(pH 3.0-4.4)" to "(pH 2.5-3.0)"; line 7, change "20-30 cc." to "10 cc." These corrections should also be made in *Methods of Analysis*, A.O.A.C. 1935, p. 347.

Insert the following results in Table 1, p. 388 of Vol. 19, *This Journal*, just preceding "Conclusions":

| Filtrate from cold trichloroacetic acid mixture heated to 50°C. + equal vol. hot (50°C.) saturated picric acid. | Clear | Very turbid | Clear | Turbid | Clear | Turbid |
|---|-------|-------------|-------|--------|-------|--------|
|   |       |             |       |        |       |        |

## CONTRIBUTED PAPERS

### AN IMPROVEMENT IN THE DETERMINATION OF LACTOSE IN MIXED FEED

By D. A. MAGRAW, L. E. COPELAND, and C. W. SIEVERT  
(American Dry Milk Institute, Inc., Chicago, Ill.)

In recent work on the determination of nonfat milk solids in bread a procedure was developed which, when applied to the determination of lactose in mixed feed, gives even more accurate results than were obtained with the procedure recently described by Magraw and Sievert.<sup>1</sup>

The modification results in decreased fermentation loss, as well as the elimination of one day's time in the procedure. This modification involves the inversion of starch and sugars with animal diastase before the fermentation, thus making it possible to complete the fermentation in 17-18 hours in place of 40-48. With this short method fermentation, temperatures are not held so low, nor controlled so closely. The only precaution necessary is to keep the fermentation temperature above 26.5°C. (80°F.). The best results were obtained at temperatures between 82 and 86°F. The former low fermentation temperature is much more difficult to control during the hot summer months. Also the short time of fermentation eliminates to a great extent the possibility for bacterial growth and enzymatic action, due to maintaining a more sterile solution at the time of fermentation, since the solutions are heated in a hot water bath.

The improved procedure follows:

#### APPARATUS AND REAGENTS

*Centrifuge and suitable sediment tubes.*

*1G4 Jena fritted-glass filtering crucible.*

*Animal diastase.<sup>2</sup>*

*Invertase-melibiose scales.<sup>3</sup>*

*Baker's yeast.*

*Solution of saturated neutral lead acetate.*

*Solution of 5% mercuric chloride.*

*Solution of 20% phosphotungstic acid.*

*Hydrogen sulfide.*

*Standard solution of sodium thiosulfate (19 grams of crystals per liter, approx. 0.005 gram Cu per cc.).*

#### DETERMINATION

Place 16.25 grams (weighed to within 0.03 gram) of the well-mixed feed in a 300 cc. volumetric flask with about 200 cc. of distilled water and digest in a hot water bath, with occasional shaking, for a period of 30 minutes. Cool, fill to volume with distilled water, and centrifuge.

Place 150 cc. of the supernatant solution in a 200 cc. volumetric flask. Add 0.25 gram of the animal diastase and place in a constant temperature bath of 52°-

<sup>1</sup> *Ind. Eng. Chem. Anal. Ed.*, 7, 106 (1935).

<sup>2</sup> Obtained from Armour & Co., Pharmaceutical Dept., Chicago, Ill.

<sup>3</sup> Nulomoline Co., 111 Wall St., New York City.

55°C. for 25–30 minutes. Place the flask in a boiling water bath for 15 minutes. Cool the flask and add another 0.25 gram of the animal diastase and repeat the inversion and subsequent heating as before. After the flask has been cooled to room temperature, add 75 mg. of the invertase-melibiose scales and 1.5–2 grams of baker's yeast and plug the flask with sterile cotton. After fermentation at 26.5° to 30°C. (80–86°F.), with a possible upper limit of 33°C. (90°F.), for a period of 17–18 hours, make up the volume and centrifuge. Reduce 190 cc. of the supernatant liquid to 25 or 50 cc. by boiling and wash into a 100 cc. volumetric flask with the aid of hot distilled water. Add 10 cc. of the saturated neutral lead acetate solution, make up to volume, and centrifuge. To 50 cc. of the clear liquid in a 100 cc. volumetric flask add 2.5 cc. of a 5% solution of mercuric chloride and allow to stand 15 minutes, with repeated shaking. Then add 5 cc. of a 20% solution of phosphotungstic acid. Make up to volume with distilled water and remove the precipitate by centrifuging. If the resulting solution is not clear after centrifuging, it should be filtered through a dry filter paper. Saturate the resulting liquid with H<sub>2</sub>S and filter. Pipet 50 cc. of the clear, colorless solution into a 400 cc. beaker, mark the level and boil to remove the H<sub>2</sub>S. Add water to restore the volume to 50 cc., and determine lactose by the Munson & Walker method. (Consistently better results are possible with a Jena fritted-glass filtering crucible, 1G4, than with an asbestos mat in a Gooch crucible.) After thoroughly washing the precipitate, dissolve in 5 cc. of hot nitric acid (1+1). Add 0.5 gram of urea<sup>1</sup> and boil for 2 minutes. Then determine the copper by titration with the standard sodium thiosulfate method. The milligrams of lactose are obtained from the Munson & Walker table.<sup>2</sup>

#### CALCULATIONS

To obtain the number of grams of dry material used in the aliquot, use Formula 1:

$$\text{Formula 1: } \frac{150}{(300-8)} \times \frac{190}{200} \times \frac{50}{100} \times \frac{50}{(100-1)} \times 16.25 = 2.00 \text{ grams}$$

These figures are the dilutions that are made in the method, and the (300–8) and (100–1) allow for a correction of 9 cc. for volumes of precipitates.

To obtain the percentage of lactose in the feed, use Formula 2:

$$\text{Formula 2: } \frac{(X-0.006)}{0.96 \times 2.00} \times 100 = \text{per cent of lactose in feed.}$$

X = grams of lactose determined, 0.006 = correction for blank, 0.96 = lactose factor for fermentation loss, and 2.00 = weight of dry material in aliquot used, derived from Formula 1.

The results obtained by the previously published<sup>3</sup> procedure with 40–48 hours' fermentation showed a high lactose loss of 10 per cent, with quite wide variations. With the method presented here and known mixtures, less loss and more consistent results were shown. This loss amounted to only 4 per cent. This lower loss might be due to a shorter time for bacterial and enzymatic action and a more nearly sterile solution at the time of fermentation.

The fermentation loss is expressed by factor 0.96 in Formula 2. The correction factor of 0.006 gram indicated in Formula 2 is a blank correc-

<sup>1</sup> Lyle O. Hill, *Ind. Eng. Chem. Anal. Ed.*, 8, 200 (1936).

<sup>2</sup> *Methods of Analysis, A.O.A.C.*, 1930, 514.

<sup>3</sup> *Loc. cit.*

tion on lactose-free samples. This correction blank was obtained from an average of many analyses of feeds containing no milk products.

This method has been used on more than 100 different feed mixes and has given satisfactory and consistent results. Results obtained on some known feeds by this method follow:

| <i>Lactose Added</i> |   | <i>per cent</i> | <i>Lactose Found</i> |
|----------------------|---|-----------------|----------------------|
|                      |   |                 | <i>per cent</i>      |
| Growing Mash         |   | 0.0             | 0.0                  |
| "                    | " | .78             | .77                  |
| "                    | " | 1.57            | 1.54                 |
| "                    | " | 1.57            | 1.60                 |
| "                    | " | 3.14            | 3.19                 |
| "                    | " | 3.14            | 3.14                 |
| Chick Mash           |   | 0.0             | 0.06                 |
| "                    | " | .62             | .53                  |
| "                    | " | 1.25            | 1.37                 |
| "                    | " | 1.88            | 1.84                 |
| "                    | " | 2.50            | 2.45                 |
| "                    | " | 3.12            | 3.16                 |
| "                    | " | 3.75            | 3.70                 |
| "                    | " | 4.37            | 4.52                 |

#### SUMMARY

An improved method for the determination of lactose in mixed feed is described.

Fermentation is stabilized, giving less fermentation loss as well as shortened fermentation time.

Results are accurate within  $\pm .20$  per cent and check determinations are precise.

#### CHEMICAL STUDIES ON MATURITY OF CANNED PEAS

By V. B. BONNEY and S. C. ROWE<sup>1</sup> (Food Division,<sup>2</sup> Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

During the summer of 1935, additional data were obtained on the composition of canned peas of different degrees of maturity to be used in interpreting the standard of quality for canned peas promulgated by the Secretary of Agriculture under the McNary-Mapes amendment to the food and drugs act. The packs, which included 9 varieties, were made in 11 different states in collaboration with the National Cannery Association laboratories, since they were making similar packs in connection with their work.<sup>3</sup>

<sup>1</sup> Thanks are due to J. I. Palmore and L. M. Beacham of the Canned Food Section of Food Division for assistance in the analytical work and to the National Cannery Association for permission to share in the experimental plots that they had set aside for making packs of peas at different stages of maturity.

<sup>2</sup> W. B. White, Chief.

<sup>3</sup> See paper, "Studies on Maturity and Canning Quality of Green Peas," by C. A. Greenleaf, presented before the Pea Section of the convention of the National Cannery Association, January, 1936 (*Canner*, 82, No. 11, Part 2, pp. 68-72 (1936); *Canning Trade*, 58, No. 28, pp. 7-12 (1936)).

In preparing these packs a plot of peas, usually an acre, was selected, and arrangements were made with the owner to permit the field to stand uncut until ready for the experimental packs. The plot was divided into 5 equal parts. When the peas had reached the stage desired for the experimental pack, one of the 5 parts was cut and vined in the factory viners. The shelled peas were cleaned and in most cases graded for size. The different sieve sizes, or, if they were not graded for size, the run-of-pod peas, were then blanched in water kept as near boiling as possible, washed in cold water, and canned in a brine made with 15 pounds of salt and 25 pounds of sugar to 100 gallons. With the exception of the packs in Illinois and one lot coded "MinnaWM," all packs were made in plain No. 2 tin cans. The Illinois packs were in C-enamel No. 2 cans, and the

TABLE 1.—*Alaska peas*

| CODE     | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|          |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|          | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| M2AB     | 3                   | 35      | 240   | 12.5             | 20.97      | 13.33             | 0                         | 0                                      | 9.12                         |
| M3AB     | 3                   | 35      | 240   | 12.5             | 20.87      | 13.48             | 0                         | 0                                      | 12.16                        |
| M4AB     | 3                   | 35      | 240   | 12.5             | 21.03      | 13.72             | 0                         | 0                                      | 13.88                        |
| M2AO     | 3                   | 35      | 240   | 12.5             | 21.41      | 12.62             | 0                         | 0                                      | 9.62                         |
| M3AO     | 3                   | 35      | 240   | 12.5             | 21.32      | 13.27             | 0                         | 0                                      | 13.41                        |
| M4AO     | 3                   | 35      | 240   | 12.5             | 21.49      | 13.11             | 0                         | 0                                      | 15.81                        |
| M2AA     | 3                   | 35      | 240   | 12.0             | 21.36      | 12.18             | 0                         | 2                                      | 12.08                        |
| M3AA     | 3                   | 35      | 240   | 12.0             | 21.65      | 12.67             | 0                         | 1                                      | 16.55                        |
| M4AA     | 3                   | 35      | 240   | 11.5             | 21.90      | 12.10             | 4                         | 13                                     | 20.47                        |
| MaAA/F   | 3                   | 35      | 240   | 12.0             | 21.89      | 12.90             | 0                         | —                                      | 24.16                        |
| MaAA/S   | 3                   | 35      | 240   | 12.0             | 22.08      | 14.00             | 7                         | —                                      | 28.28                        |
| M2AM     | 4                   | 35      | 240   | 12.0             | 21.47      | 12.62             | 1                         | 5                                      | 12.22                        |
| M3AM     | 4                   | 35      | 240   | 12.0             | 21.93      | 13.32             | 4                         | 46                                     | 22.32                        |
| M4AM     | 4                   | 35      | 240   | 12.0             | 22.12      | 13.50             | 24                        | 49                                     | 23.62                        |
| M2AD     | 6                   | 35      | 240   | 11.0             | 22.48      | 16.30             | 24                        | 94                                     | 26.78                        |
| M3AD     | 6                   | 35      | 240   | 11.0             | 22.36      | 14.92             | 67                        | 94                                     | 26.71                        |
| M4AD     | 6                   | 35      | 240   | 11.0             | 22.33      | 13.85             | 59                        | 72                                     | 26.27                        |
| MaAD     | 6                   | 35      | 240   | 11.0             | 22.30      | 14.50             | 56                        | 62                                     | 25.60                        |
| MaAD/S   | 10                  | 35      | 240   | 10.0             | 22.19      | 13.75             | 63                        | 95                                     | 24.38                        |
| MaAD/F   | 6                   | 35      | 240   | 11.0             | 22.33      | 13.28             | 22                        | 65                                     | 24.71                        |
| MaAD/S9  | 10                  | 35      | 240   | 9.0              | 21.96      | 13.03             | 71                        | 94                                     | 25.56                        |
| MaAD/S11 | 10                  | 35      | 240   | 11.0             | 22.34      | 15.74             | 75                        | 91                                     | 24.67                        |
| U2AB     | 4                   | 25      | 248   | 12.0             | 21.29      | 12.44             | 0                         | 0                                      | 10.94                        |
| U3AB     | 4                   | 25      | 248   | 12.0             | 21.44      | 12.68             | 0                         | 0                                      | 14.78                        |
| U4AB     | 4                   | 25      | 248   | 12.0             | 21.44      | 13.03             | 0                         | Trace                                  | 18.14                        |
| UaAB/S   | 4                   | 25      | 248   | 12.0             | 21.52      | 12.77             | 0                         | 12                                     | 20.70                        |
| U2AO     | 4                   | 25      | 248   | 12.0             | 21.31      | 12.44             | 0                         | 0                                      | 11.90                        |
| U3AO     | 4                   | 25      | 248   | 12.0             | 21.52      | 12.87             | 0                         | 0                                      | 16.05                        |
| U4AO     | 4                   | 25      | 248   | 12.0             | 21.62      | 12.72             | 0                         | 9                                      | 19.53                        |

TABLE 1.—(Continued)

| CODE   | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|--------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|        |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|        | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| UaAO/S | 4                   | 25      | 248   | 12.0             | 21.77      | 13.08             | 3                         | 33                                     | 22.91                        |
| U2AA   | 4                   | 25      | 248   | 12.0             | 21.06      | 12.35             | 0                         | 1                                      | 12.42                        |
| U3AA   | 4                   | 25      | 248   | 12.0             | 21.55      | 12.56             | 2                         | 3                                      | 16.46                        |
| U4AA   | 4                   | 25      | 248   | 12.0             | 21.71      | 12.75             | 2                         | 13                                     | 20.52                        |
| UaAA/S | 4                   | 25      | 248   | 12.0             | 21.75      | 12.75             | 4                         | 24                                     | 21.09                        |
| U2AM   | 4                   | 28      | 248   | 12.0             | 21.09      | 12.96             | 5                         | 12                                     | 19.56                        |
| U3AM   | 4                   | 28      | 248   | 12.0             | 21.84      | 13.40             | 16                        | 37                                     | 22.54                        |
| U4AM   | 4                   | 28      | 248   | 12.0             | 20.69      | 13.46             | 13                        | 62                                     | 24.18                        |
| UaAM/S | 6                   | 28      | 248   | 11.0             | 21.97      | 12.50             | 20                        | 62                                     | 23.98                        |
| U4AM/S | 6                   | 28      | 248   | 11.0             | 21.74      | 14.28             | —                         | 82                                     | 25.07                        |
| UaAD   | 8                   | 30      | 248   | 10.5             | 22.05      | 16.57             | 52                        | 92                                     | 26.70                        |
| UaAD/S | 10                  | 30      | 248   | 10.0             | 21.63      | 14.21             | 66                        | 93                                     | 25.14                        |
| UaAD/F | 8                   | 30      | 248   | 11.0             | 21.97      | 14.12             | 48                        | 75                                     | 24.19                        |
| W1AB   | 3                   | 35      | 240   | 12.0             | 21.39      | 11.79             | 0                         | 0                                      | 9.07                         |
| W2AB   | 3                   | 35      | 240   | 12.0             | 21.20      | 11.83             | 0                         | 0                                      | 11.75                        |
| W3AB   | 3                   | 35      | 240   | 12.0             | 21.83      | 11.85             | 0                         | 1                                      | 14.75                        |
| W2AA   | 4                   | 35      | 240   | 11.5             | 21.82      | 11.61             | 0                         | 10                                     | 15.58                        |
| W3AA   | 4                   | 35      | 240   | 11.5             | 21.94      | 11.70             | 0                         | 3                                      | 19.34                        |
| W4AA   | 4                   | 35      | 240   | 11.0             | 21.03      | 11.23             | 0                         | 6                                      | 20.58                        |
| W5AA   | 4                   | 35      | 240   | 11.0             | 22.03      | 11.37             | 0                         | 10                                     | 20.66                        |
| W2AM   | 4                   | 35      | 240   | 11.0             | 22.06      | 11.58             | 0                         | 46                                     | 22.54                        |
| W3AM   | 4                   | 35      | 240   | 11.0             | 21.66      | 11.62             | 0                         | 56                                     | 24.31                        |
| W4AM   | 4                   | 35      | 240   | 10.5             | 21.45      | 10.88             | 3                         | 44                                     | 24.49                        |
| W5AM   | 4                   | 35      | 240   | 10.5             | 22.08      | 11.02             | 2                         | 42                                     | 24.78                        |
| W2AD   | 7                   | 35      | 240   | 9.0              | 20.94      | 11.33             | 35                        | 72                                     | 25.40                        |
| W3AD   | 7                   | 35      | 240   | 9.0              | 20.43      | 10.89             | 54                        | 79                                     | 26.11                        |
| W4AD   | 7                   | 35      | 240   | 9.0              | 21.24      | 10.95             | 58                        | 76                                     | 25.72                        |
| W5AD   | 7                   | 35      | 240   | 9.0              | 21.83      | 10.45             | 36                        | 50                                     | 22.09                        |
| I2AB   | 4                   | 35      | 240   | 12.0             | 21.13      | 12.53             | 0                         | 0                                      | 9.18                         |
| I3AB   | 4                   | 35      | 240   | 12.0             | 21.37      | 12.69             | 0                         | 0                                      | 11.53                        |
| I4AB   | 4                   | 35      | 240   | 12.0             | 21.32      | 12.78             | 0                         | 0                                      | 14.22                        |
| I5AB   | 4                   | 35      | 240   | 12.0             | 21.68      | 12.59             | 0                         | 0                                      | 15.19                        |
| I2AO   | 4                   | 35      | 240   | 12.0             | 21.11      | 12.49             | 0                         | 0                                      | 10.01                        |
| I3AO   | 4                   | 35      | 240   | 12.0             | 21.58      | 12.50             | 0                         | 0                                      | 13.11                        |
| I4AO   | 4                   | 35      | 240   | 12.0             | 21.79      | 13.07             | 0                         | 0                                      | 16.66                        |
| I5AO   | 4                   | 35      | 240   | 12.0             | 21.89      | 13.11             | 0                         | 0                                      | 17.08                        |
| I2AA   | 4                   | 35      | 240   | 12.0             | 21.46      | 12.58             | 0                         | 0                                      | 11.27                        |
| I3AA   | 4                   | 35      | 240   | 12.0             | 21.28      | 12.56             | 0                         | 0                                      | 16.26                        |
| I4AA   | 4                   | 35      | 240   | 12.0             | 21.82      | 12.46             | 2                         | 3                                      | 19.36                        |
| I5AA   | 4                   | 35      | 240   | 12.0             | 21.73      | 12.68             | 2                         | 12                                     | 21.36                        |
| I3AAWB | 4                   | 35      | 240   | 12.0             | 21.84      | 12.75             | 0                         | 1                                      | 15.85                        |
| I3AA12 | 12                  | 35      | 240   | 12.0             | 21.67      | 12.25             | 0                         | 0                                      | 16.67                        |
| I3AA6H | 4                   | 35      | 240   | 12.0             | 21.43      | 12.52             | 1                         | 2                                      | 16.83                        |



TABLE 1.—(Continued)

| CODE      | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INOL.<br>SOLIDS |
|-----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|-----------------------------|
|           |                     | TIME    | TEMP. |                  |            |                   |                           |  |                             |
|           | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                    |
| I2AM      | 4                   | 35      | 240   | 12.0             | 21.85      | 12.68             | 0                         | 12                                     | 21.27                       |
| I3AM      | 4                   | 35      | 240   | 11.5             | 21.73      | 12.76             | 5                         | 39                                     | 23.42                       |
| I4AM      | 6                   | 35      | 240   | 11.0             | 21.73      | 12.97             | 13                        | 56                                     | 25.09                       |
| I5AM      | 6                   | 35      | 240   | 10.5             | 22.02      | 12.84             | 43                        | 54                                     | 25.16                       |
| IaAM      | 6                   | 35      | 240   | 11.0             | 21.85      | 12.49             | 22                        | 35                                     | 23.48                       |
| I2AD      | 4                   | 35      | 240   | 10.5             | 21.38      | 11.68             | 3                         | 30                                     | 23.19                       |
| I3AD      | 4                   | 35      | 240   | 10.5             | 21.59      | 12.35             | 18                        | 73                                     | 25.01                       |
| I4AD      | 6                   | 35      | 240   | 10.5             | 21.98      | 12.63             | 46                        | 67                                     | 24.97                       |
| I5AD      | 6                   | 35      | 240   | 10.5             | 21.91      | 12.97             | 62                        | 59                                     | 25.08                       |
|           |                     |         |       |                  |            |                   |                           |  |                             |
| E1AB      | 3                   | 35      | 240   | 12.5             | 21.06      | 12.47             | 0                         | 0                                      | 7.57                        |
| E2AB      | 3                   | 35      | 240   | 12.5             | 20.69      | 12.35             | 0                         | 0                                      | 11.15                       |
| E3AB      | 4                   | 35      | 240   | 12.5             | 20.65      | 12.61             | 0                         | 0                                      | 14.53                       |
| E4AB      | 4                   | 35      | 240   | 12.5             | 21.01      | 12.49             | 0                         | 0                                      | 16.46                       |
| E1ABB     | 3                   | 35      | 240   | 12.5             | 20.54      | 12.63             | 0                         | 0                                      | 7.34                        |
| E2ABB     | 3                   | 35      | 240   | 12.5             | 20.39      | 12.25             | 0                         | 0                                      | 10.07                       |
| E3ABB     | 4                   | 35      | 240   | 12.5             | 20.91      | 12.46             | 0                         | 0                                      | 14.08                       |
| E4ABB     | 4                   | 35      | 240   | 12.5             | 21.48      | 12.42             | 0                         | 0                                      | 16.91                       |
| E1AO      | 3                   | 35      | 240   | 12.5             | 20.68      | 12.28             | 0                         | 0                                      | 8.13                        |
| E2AO      | 3                   | 35      | 240   | 12.5             | 21.07      | 12.42             | 0                         | 0                                      | 11.28                       |
| E3AO      | 4                   | 35      | 240   | 12.5             | 21.03      | 12.56             | 0                         | 0                                      | 16.86                       |
| E4AO      | 4                   | 35      | 240   | 12.5             | 20.89      | 12.85             | 0                         | 0                                      | 19.41                       |
| E1AA      | 3                   | 35      | 240   | 12.0             | 20.30      | 12.12             | 0                         | 0                                      | 8.82                        |
| E2AA      | 3                   | 35      | 240   | 12.0             | 21.42      | 12.34             | 0                         | 0                                      | 12.54                       |
| E3AA      | 4                   | 35      | 240   | 12.0             | 21.06      | 12.42             | 0                         | Trace                                  | 18.56                       |
| E4AA      | 4                   | 35      | 240   | 11.5             | 21.54      | 12.08             | 0                         | 5                                      | 21.26                       |
| E2AM      | 4                   | 35      | 240   | 12.0             | 21.39      | 13.19             | 0                         | 11                                     | 18.38                       |
| E3AM      | 4                   | 35      | 240   | 11.5             | 21.30      | 11.88             | 1                         | 34                                     | 22.90                       |
| E4AM      | 4                   | 35      | 240   | 11.0             | 21.59      | 11.79             | 6                         | —                                      | 25.19                       |
| E2AD      | 4                   | 35      | 240   | 10.0             | 20.65      | 11.30             | 8                         | 27                                     | 24.39                       |
| E3AD      | 4                   | 35      | 240   | 10.0             | 20.30      | 11.62             | 15                        | 34                                     | 25.39                       |
| E4AD      | 4                   | 35      | 240   | 9.5              | 20.08      | 11.27             | 43                        | —                                      | 25.34                       |
|           |                     |         |       |                  |            |                   |                           |  |                             |
| N3AB      | 3                   | 35      | 240   | 12.5             | 21.26      | 12.78             | 0                         | 0                                      | 11.14                       |
| N4AB      | 3                   | 35      | 240   | 12.5             | 21.45      | 12.59             | 0                         | 0                                      | 14.24                       |
| N3AA      | 3                   | 35      | 240   | 12.0             | 21.03      | 11.82             | 0                         | 5                                      | 16.19                       |
| N4AA      | 3                   | 35      | 240   | 12.0             | 21.04      | 12.37             | 0                         | Trace                                  | 19.41                       |
| N3AM      | 3                   | 35      | 240   | 11.75            | 20.72      | 12.12             | 0                         | 23                                     | 23.65                       |
| N4AM      | 3                   | 35      | 240   | 11.75            | 21.01      | 12.09             | 0                         | 59                                     | 25.01                       |
| NaAM/S11½ | 3                   | 35      | 240   | 11.25            | 20.63      | 11.70             | 1                         | 51                                     | 24.47                       |
| NaAM/F11½ | 3                   | 35      | 240   | 11.75            | 20.65      | 12.43             | 0                         | 8                                      | 21.86                       |
| N3AD      | 6                   | 35      | 240   | 11.0             | 19.28      | 11.84             | 0                         | 81                                     | 26.54                       |
| N4AD      | 6                   | 35      | 240   | 11.0             | 21.81      | 11.65             | 1                         | 95                                     | 27.95                       |
| NaAD      | 6                   | 35      | 240   | 11.0             | 20.66      | 11.55             | 0                         | 87                                     | 27.28                       |
| NaADS10½  | 6                   | 35      | 240   | 10.5             | 21.02      | 11.18             | 3                         | 91                                     | 27.58                       |

TABLE 1.—(Continued)

| CODE     | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|          |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|          | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| NaADS10  | 6                   | 35      | 240   | 10.0             | 21.46      | 10.95             | 2                         | 85                                     | 27.62                        |
| NaADS11  | 6                   | 35      | 240   | 11.0             | 20.84      | 12.00             | 1                         | 90                                     | 27.41                        |
| NaADF10½ | 6                   | 35      | 240   | 10.5             | 20.75      | 11.19             | 0                         | 53                                     | 25.93                        |
| NaADF11½ | 6                   | 35      | 240   | 11.5             | 20.70      | 12.07             | 0                         | 65                                     | 26.38                        |
| NaADF    | 6                   | 35      | 240   | 11.0             | 20.74      | 11.65             | 0                         | 69                                     | 26.46                        |
| Minn1AB  | 4                   | 35      | 240   | 13.5             | 21.06      | 13.60             | 0                         | 0                                      | 7.10                         |
| Minn2AB  | 5                   | 35      | 240   | 13.5             | 21.38      | 13.19             | 0                         | 0                                      | 8.46                         |
| Minn3AB  | 7                   | 35      | 240   | 13.5             | —          | —                 | —                         | —                                      | —                            |
| Minn1ABB | 4                   | 35      | 240   | 13.5             | 21.33      | 13.60             | 0                         | 0                                      | 7.73                         |
| Minn2ABB | 5                   | 35      | 240   | 13.5             | 21.10      | 13.29             | 0                         | 0                                      | 9.70                         |
| Minn3ABB | 6                   | 35      | 240   | 13.25            | 21.13      | 12.82             | 0                         | 0                                      | 12.07                        |
| Minn4ABB | 7                   | 35      | 240   | 13.0             | 21.40      | 12.30             | 0                         | 0                                      | 14.85                        |
| Minn1AO  | 4                   | 35      | 240   | 13.5             | 21.46      | 13.25             | 0                         | 0                                      | 8.27                         |
| Minn2AO  | 5                   | 35      | 240   | 13.5             | 21.57      | 13.18             | 0                         | 0                                      | 11.24                        |
| Minn3AO  | 6                   | 35      | 240   | 13.0             | 21.51      | 12.70             | 0                         | 0                                      | 15.48                        |
| Minn4AO  | 7                   | 35      | 240   | 12.75            | 21.09      | 12.62             | 0                         | 0                                      | 18.31                        |
| Minn1AA  | 4                   | 35      | 240   | 13.25            | 21.09      | 12.81             | 0                         | 0                                      | 8.80                         |
| Minn2AA  | 5                   | 35      | 240   | 13.0             | 21.24      | 12.58             | 0                         | 0                                      | 11.40                        |
| Minn3AA  | 7                   | 35      | 240   | 12.5             | 21.55      | 12.12             | 0                         | 0                                      | 17.03                        |
| Minn4AA  | 8                   | 35      | 240   | 12.25            | 21.68      | 12.05             | 0                         | 0                                      | 20.06                        |
| Minn1AM  | 4                   | 35      | 240   | 13.0             | 21.31      | 13.00             | —                         | —                                      | 10.44                        |
| Minn2AM  | 5                   | 35      | 240   | 12.5             | 21.46      | 12.44             | 0                         | 0                                      | 15.34                        |
| Minn3AM  | 7                   | 35      | 240   | 12.0             | 21.39      | 12.35             | 0                         | 6                                      | 20.07                        |
| Minn4AM  | 8                   | 35      | 240   | 11.75            | 21.23      | 12.56             | 1                         | 17                                     | 22.83                        |
| Minn5AM  | 9                   | 35      | 240   | 11.25            | 21.83      | 11.90             | 7                         | 25                                     | 23.06                        |
| Minn2AD  | 5                   | 35      | 240   | 12.5             | 21.64      | 12.32             | 0                         | 10                                     | 17.06                        |
| Minn3AD  | 7                   | 35      | 240   | 12.0             | 21.96      | 12.09             | 0                         | 39                                     | 23.26                        |
| Minn4AD  | 8                   | 35      | 240   | 11.75            | 22.14      | 11.91             | 0                         | 62                                     | 25.23                        |
| Minn5AD  | 9                   | 35      | 240   | 11.25            | 22.11      | 11.65             | 5                         | 62                                     | 25.69                        |
| BaAO     | 3                   | 35      | 240   | 12.0             | 21.77      | 12.52             | 0                         | Trace                                  | 15.78                        |
| BaAA     | 3                   | 35      | 240   | 11.5             | 21.53      | 11.22             | 0                         | 3                                      | 17.85                        |
| BaAM     | 4                   | 35      | 240   | 11.5             | 22.10      | 12.25             | 7                         | 54                                     | 23.92                        |
| BaAD     | 6                   | 35      | 240   | 10.5             | 20.35      | 12.37             | 26                        | 52                                     | 24.55                        |
| BaADS10  | 10                  | 35      | 240   | 10.0             | 22.11      | 12.90             | 54                        | 55                                     | 23.20                        |
| BaADS9   | 10                  | 35      | 240   | 9.0              | 21.67      | 11.54             | 59                        | 59                                     | 22.64                        |
| BaADF11  | 6                   | 35      | 240   | 11.0             | 22.22      | 13.25             | 11                        | 40                                     | 23.63                        |
| BaADF10½ | 6                   | 35      | 240   | 10.5             | 22.25      | 12.53             | 8                         | 40                                     | 23.52                        |
| PaADS10  | 10                  | 35      | 240   | 10.0             | 21.44      | 13.26             | 50                        | 91                                     | 26.16                        |
| PaADF11  | 6                   | 35      | 240   | 11.0             | 21.43      | 14.31             | 17                        | 88                                     | 26.10                        |
| PaADF10½ | 6                   | 35      | 240   | 10.5             | 20.88      | 13.50             | 16                        | 92                                     | 26.44                        |
| PaAD     | 8                   | 35      | 240   | 10.5             | 20.42      | 13.61             | 38                        | 91                                     | 26.09                        |
| DAAD     | 4                   | 40      | 242   | 9.0              | 21.03      | 11.58             | 40                        | 59                                     | 23.74                        |

"MinnaWM" pack was in a plain 303 can. Portions of each of the packs were brought to the Food Division laboratory in Washington for analysis. The packs were coded, with four or more letters on each can. The code marks have been used to identify the results given in Tables 1 and 2. The first letter of the code shows the locality where the peas were grown, as follows:

B—Bridgeville, Del.; D—Dayton, Wash.; E—Eureka, Ill.; H—Hartland, Maine; I—Circleville, Ohio; M—Walkerton, Va.; Minn—Le Sueur, Minn.; N—Geneva, N. Y.; P—Hanover, Pa.; U—Murray, Utah; W—Waunakee, Wis.

The second letter shows the sieve size, *i.e.*, 2, 3, 4, 5, or 6. The letter "a" designates "ungraded" for size. The third letter shows the variety of the peas:

A—Alaska, G—Green Giant, H—Horsford, L—Thomas Laxton, P—Perfection, S—Surprise, X—Senator (if grown in Washington), Wisconsin Early Sweet (if grown in Pennsylvania), W—Prince of Wales.

The fourth letter shows the state of maturity at which the peas were harvested. The judgment of the grower or of the cannery's field man, or both, was taken into consideration in each instance in determining the maturity of the field.—The meaning of each letter follows:

*B—Before Optimum.*—As nearly as could be determined, this stage was at least a day before the peas were ready for canning. In general, all the vines and pods were green. The pods were starting to fill, but in most cases were not as yet completely filled.

On certain of the "E" and "Minn" packs of Alaska peas there appears a fifth letter, "B". In both of these packs peas were cut twice before reaching canning optimum, the fifth letter "B" indicating that it was the second cutting before optimum.

*O—Optimum.*—The peas for this pack were judged to be at just the right stage for canning for highest quality. All the vines and pods were green and most of the pods fairly well filled. In few, if any, pods were the peas extremely tight.

*A—After Optimum.*—The peas for this pack were taken from vines which indicated the peas to be a little past the best canning stage. The vines were still green, although possibly showing some yellowing at the base. The pods were green and very well filled. Few, if any, of the pods were as yet showing any wrinkling.

In the "Minn" packs of the Prince of Wales variety appears the code mark "Minna WMA." This pack was from a cutting of the peas between the "after optimum" and the "mature" stage.

*M—Mature.*—The peas for this pack were taken from vines which were considered about as old as could be properly canned as standard green peas. Most of the vines were still green, but some yellow was showing at the base and some of the vines were almost completely dried. All the pods were very full, many of them light colored and wrinkled, but very few dry yellow pods were present.

*D—Distinctly Mature.*—The peas for this pack were obtained after the vines had begun to show considerable drying. At least 10 per cent of the pods were dry and yellow. With Alaskas, when shelled, about the same percentage of gray peas were found as there were dry yellow pods. In making these experimental packs of the Alaska variety gray peas were found only in yellow pods. With certain varieties, notably Senators, a dry yellow pod did not necessarily mean an over-mature pea. The D series of Senators packed at Dayton, Wash., was obtained from a field in

TABLE 2.—*Sweet peas*

| CODE     | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|          |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|          | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| N3PO     | 3                   | 35      | 240   | 12.5             | 19.78      | 12.15             | 0                         | 0                                      | 9.76                         |
| N4PO     | 3                   | 35      | 240   | 12.5             | 21.08      | 12.32             | 0                         | 0                                      | 11.41                        |
| N5PO     | 3                   | 35      | 240   | 12.5             | 21.10      | 12.51             | 0                         | 0                                      | 12.12                        |
| N3PA     | 3                   | 35      | 240   | 12.5             | 20.58      | 12.58             | 0                         | 0                                      | 15.30                        |
| N4PA     | 3                   | 35      | 240   | 12.5             | 21.14      | 12.75             | 0                         | 0                                      | 16.80                        |
| N5PA     | 3                   | 35      | 240   | 12.5             | 20.94      | 12.69             | 0                         | 0                                      | 17.20                        |
| N3PM     | 4                   | 35      | 240   | 11.25            | 21.29      | 11.88             | 0                         | 1                                      | 18.03                        |
| N4PM     | 4                   | 35      | 240   | 11.25            | 20.71      | 12.15             | 0                         | 0                                      | 19.54                        |
| N5PM     | 4                   | 35      | 240   | 11.25            | 20.96      | 11.80             | 0                         | 0                                      | 20.84                        |
| N3PD     | 6                   | 35      | 240   | 11.0             | 21.26      | 12.18             | 0                         | 0                                      | 18.26                        |
| N4PD     | 6                   | 35      | 240   | 11.0             | 21.56      | 12.24             | 0                         | 1                                      | 20.17                        |
| N5PD     | 6                   | 35      | 240   | 11.0             | 21.29      | 11.70             | 0                         | 1                                      | 22.27                        |
| NaPD     | 6                   | 35      | 240   | 11.0             | 20.78      | 11.96             | 0                         | 0                                      | 20.36                        |
| NaPDS10½ | 6                   | 35      | 240   | 10.5             | 21.09      | 11.62             | 0                         | 0                                      | 21.41                        |
| NaPDS10  | 6                   | 35      | 240   | 10.0             | 21.34      | 11.28             | 0                         | 3                                      | 21.20                        |
| NAPDF    | 6                   | 35      | 240   | 11.0             | 21.12      | 11.90             | 0                         | 0                                      | 18.72                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| D2PB     | 4                   | 40      | 242   | 12.0             | 20.71      | 12.00             | 0                         | 0                                      | 7.62                         |
| D3PB     | 4                   | 40      | 242   | 12.0             | 19.99      | 11.86             | 0                         | 0                                      | 8.84                         |
| D4PB     | 4                   | 40      | 242   | 12.0             | 20.56      | 11.90             | 0                         | 0                                      | 10.60                        |
| D5PB     | 4                   | 40      | 242   | 12.0             | 20.96      | 12.22             | 0                         | 0                                      | 13.14                        |
| D3PO     | 4                   | 40      | 242   | 12.0             | 20.83      | 12.09             | 0                         | 0                                      | 9.13                         |
| D4PO     | 4                   | 40      | 242   | 12.0             | 20.84      | 12.14             | 0                         | 0                                      | 11.04                        |
| D5PO     | 4                   | 40      | 242   | 12.0             | 21.08      | 12.62             | 0                         | 0                                      | 13.60                        |
| D3PA     | 4                   | 40      | 242   | 12.0             | 20.80      | 12.26             | 0                         | 0                                      | 9.12                         |
| D4PA     | 4                   | 40      | 242   | 12.0             | 21.00      | 11.96             | 0                         | 0                                      | 11.84                        |
| D5PA     | 4                   | 40      | 242   | 12.0             | 21.00      | 12.47             | 0                         | 0                                      | 14.60                        |
| D3PM     | 4                   | 40      | 242   | 12.0             | 21.07      | 12.25             | 0                         | 0                                      | 12.36                        |
| D4PM     | 4                   | 40      | 242   | 12.0             | 21.15      | 12.18             | 0                         | 0                                      | 15.22                        |
| D5PM     | 4                   | 40      | 242   | 12.0             | 21.26      | 12.75             | 0                         | 0                                      | 17.52                        |
| D6PM     | 4                   | 40      | 242   | 12.0             | 21.30      | 12.43             | 0                         | 0                                      | 18.56                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| U2PB     | 4                   | 22      | 248   | 12.0             | 21.26      | 12.25             | 0                         | 0                                      | 6.77                         |
| U3PB     | 4                   | 22      | 248   | 12.0             | 21.32      | 11.92             | 0                         | 0                                      | 7.89                         |
| U4PB     | 4                   | 22      | 248   | 12.0             | 21.31      | 12.35             | 0                         | 0                                      | 9.66                         |
| U5PB     | 4                   | 22      | 248   | 12.0             | 21.40      | 12.50             | 0                         | 0                                      | 11.29                        |
| U6PB     | 4                   | 22      | 248   | 12.0             | 21.35      | 12.35             | 0                         | 0                                      | 12.91                        |
| UaPB/S   | 4                   | 22      | 248   | 12.0             | 21.46      | 12.40             | 0                         | 0                                      | 11.36                        |
| U2PO     | 4                   | 22      | 248   | 12.0             | 20.96      | 12.20             | 0                         | 0                                      | 6.65                         |
| U3PO     | 4                   | 22      | 248   | 12.0             | 21.27      | 12.58             | 0                         | 0                                      | 7.67                         |
| U4PO     | 4                   | 22      | 248   | 12.0             | 21.37      | 12.42             | 0                         | 0                                      | 9.53                         |
| U5PO     | 4                   | 22      | 248   | 12.0             | 20.96      | 12.16             | 0                         | 0                                      | 12.17                        |
| U6PO     | 4                   | 22      | 248   | 12.0             | 21.51      | 12.42             | 0                         | 0                                      | 13.44                        |
| UaPO/S   | 4                   | 22      | 248   | 12.0             | 21.54      | 12.78             | 0                         | 0                                      | 14.46                        |
| U2PA     | 4                   | 22      | 248   | 12.0             | 21.57      | 12.70             | 0                         | 0                                      | 7.12                         |

TABLE 2.—(Continued)

| CODE     | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|          |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|          | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| U3PA     | 4                   | 22      | 248   | 12.0             | 21.27      | 12.15             | 0                         | 0                                      | 8.30                         |
| U4PA     | 4                   | 22      | 248   | 12.0             | 21.43      | 12.20             | 0                         | 0                                      | 10.83                        |
| U5PA     | 4                   | 22      | 248   | 12.0             | 21.35      | 12.42             | 0                         | 0                                      | 13.40                        |
| U6PA     | 4                   | 22      | 248   | 12.0             | 21.55      | 12.53             | 0                         | 0                                      | 15.89                        |
| UaPA/S   | 4                   | 22      | 248   | 12.0             | 21.43      | 12.50             | 0                         | 0                                      | 13.86                        |
| U4PA/S   | 4                   | 22      | 248   | 12.0             | 21.49      | 12.41             | 0                         | 0                                      | 11.98                        |
| U2PM     | 4                   | 22      | 248   | 12.0             | 20.88      | 12.35             | 0                         | 0                                      | 8.52                         |
| U3PM     | 4                   | 22      | 248   | 11.5             | 21.37      | 11.90             | 0                         | 0                                      | 10.41                        |
| U4PM     | 4                   | 22      | 248   | 11.5             | 21.38      | 11.83             | 0                         | 0                                      | 12.48                        |
| U5PM     | 4                   | 22      | 248   | 11.5             | 21.45      | 12.29             | 0                         | 0                                      | 16.86                        |
| U6PM     | 4                   | 22      | 248   | 11.5             | 21.61      | 12.19             | 0                         | 0                                      | 18.13                        |
| UaPM/S   | 6                   | 22      | 248   | 11.0             | 21.43      | 12.09             | 1                         | 6                                      | 17.48                        |
| U4PM/S   | 6                   | 22      | 248   | 11.0             | 21.39      | 12.29             | 1                         | 7                                      | 17.82                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| W2PO     | 3                   | 35      | 240   | 12.0             | 21.32      | 12.14             | 0                         | 0                                      | 8.34                         |
| W3PO     | 3                   | 35      | 240   | 12.0             | 21.24      | 12.20             | 0                         | 0                                      | 10.30                        |
| W4PO     | 3                   | 35      | 240   | 12.0             | 21.28      | 12.57             | 0                         | 6                                      | 13.74                        |
| W5PO     | 3                   | 35      | 240   | 12.0             | 21.78      | 12.78             | 0                         | 8                                      | 14.90                        |
| W3PM     | 3                   | 35      | 240   | 11.0             | 21.46      | 11.96             | 0                         | 2                                      | 15.36                        |
| W4PM     | 3                   | 35      | 240   | 11.0             | 21.75      | 11.75             | 0                         | 0                                      | 16.51                        |
| W5PM     | 3                   | 35      | 240   | 11.0             | 21.07      | 11.42             | 0                         | 0                                      | 19.13                        |
| W6PM     | 3                   | 35      | 240   | 11.0             | 21.06      | 11.43             | 0                         | 0                                      | 19.92                        |
| W3PD     | 5                   | 35      | 240   | 10.0             | 21.82      | 11.65             | 0                         | 0                                      | 19.51                        |
| W4PD     | 5                   | 35      | 240   | 10.0             | 21.55      | 11.00             | 0                         | Trace                                  | 20.16                        |
| W5PD     | 5                   | 35      | 240   | 10.0             | 21.06      | 10.62             | 0                         | Trace                                  | 21.72                        |
| W6PD     | 5                   | 35      | 240   | 10.0             | 20.97      | 10.50             | 0                         | Trace                                  | 22.14                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| MinnaWO  | 6                   | 35      | 240   | 13.5             | 19.06      | 13.88             | 0                         | 0                                      | 10.03                        |
| MinnaWA  | 6                   | 40      | 240   | 13.5             | 21.49      | 14.16             | 0                         | 0                                      | 11.28                        |
| MinnaWMA | 6                   | 35      | 240   | 13.5             | 21.21      | 13.90             | 0                         | 0                                      | 12.63                        |
| MinnaWM  | 7                   | 35      | 240   | 10.75            | 17.66      | 11.25             | 0                         | 0                                      | 13.72                        |
| MinnaWD  | 7                   | 35      | 240   | 12.75            | 21.69      | 13.75             | 0                         | 0                                      | 19.10                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| PaXB     | 3                   | 35      | 240   | 12.5             | 21.37      | 12.83             | 0                         | 0                                      | 9.73                         |
| PaXO     | 3                   | 35      | 240   | 12.0             | 20.70      | 12.44             | 0                         | 0                                      | 10.71                        |
| PaXA     | 3                   | 35      | 240   | 11.5             | 20.78      | 12.20             | 0                         | 0                                      | 13.26                        |
| PaXM     | 4                   | 35      | 240   | 11.25            | 20.96      | 11.78             | 0                         | 0                                      | 16.26                        |
| PaXD     | 5                   | 35      | 240   | 11.0             | 20.68      | 11.74             | 0                         | 17                                     | 23.31                        |
| PaXDS11  | 6                   | 35      | 240   | 11.0             | 20.36      | 12.06             | 0                         | 35                                     | 23.36                        |
| PaXDS10½ | 6                   | 35      | 240   | 10.5             | 20.77      | 11.33             | 0                         | 34                                     | 23.07                        |
| PaXDF11  | 4                   | 35      | 240   | 11.0             | 21.11      | 11.67             | 0                         | 15                                     | 23.01                        |
|          |                     |         |       |                  |            |                   |                           |  |                              |
| D5XB     | 4                   | 40      | 242   | 12.0             | 20.18      | 12.21             | 0                         | 0                                      | 12.02                        |
| D6XB     | 4                   | 40      | 242   | 12.0             | 20.29      | 12.50             | 0                         | 0                                      | 14.40                        |
| D7XB     | 4                   | 40      | 242   | 12.0             | 20.72      | 12.62             | 0                         | 0                                      | 16.29                        |

TABLE 2.—(Continued)

| CODE     | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INSOL.<br>SOLIDS |
|----------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|------------------------------|
|          |                     | TIME    | TEMP. |                  |            |                   |                           |  |                              |
|          | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                     |
| D5XO     | 4                   | 40      | 242   | 12.0             | 20.92      | 12.12             | 0                         | 0                                      | 12.07                        |
| D6XO     | 4                   | 40      | 242   | 12.0             | 20.80      | 12.11             | 0                         | 0                                      | 14.96                        |
| D7XO     | 4                   | 40      | 242   | 12.0             | 20.97      | 12.39             | 0                         | 0                                      | 16.68                        |
| D5XA     | 4                   | 40      | 242   | 12.0             | 21.23      | 12.70             | 0                         | 0                                      | 16.04                        |
| D6XA     | 4                   | 40      | 242   | 12.0             | 21.19      | 12.55             | 0                         | 0                                      | 18.33                        |
| D7XA     | 4                   | 40      | 242   | 12.0             | 21.32      | 12.64             | 0                         | 0                                      | 18.88                        |
| D5XD     | 4                   | 40      | 242   | 12.0             | 21.10      | 12.50             | 0                         | 0                                      | 15.44                        |
| D6XD     | 4                   | 40      | 242   | 12.0             | 21.16      | 12.88             | 0                         | 0                                      | 17.74                        |
| D7XD     | 4                   | 40      | 242   | 12.0             | 21.34      | 13.16             | 0                         | 0                                      | 18.89                        |
| H2SB     | 3                   | 35      | 240   | 12.5             | 20.08      | 12.04             | 0                         | 0                                      | 8.13                         |
| H3SB     | 3                   | 35      | 240   | 12.5             | 20.59      | 12.92             | 0                         | 0                                      | 10.45                        |
| H4SB     | 3                   | 35      | 240   | 12.5             | 20.47      | 12.00             | 0                         | 0                                      | 11.89                        |
| H2SO     | 3                   | 35      | 240   | 12.5             | 20.66      | 12.61             | 0                         | 0                                      | 12.29                        |
| H3SO     | 3                   | 35      | 240   | 12.5             | 20.94      | 13.09             | 0                         | 0                                      | 10.82                        |
| H4SO     | 3                   | 35      | 240   | 12.5             | 20.59      | 13.10             | 0                         | 0                                      | 12.48                        |
| H5SO     | 3                   | 35      | 240   | 12.5             | 20.03      | 13.03             | 0                         | 0                                      | 14.33                        |
| H2SA     | 3                   | 35      | 240   | 12.5             | 20.00      | 13.46             | 0                         | 0                                      | 12.60                        |
| H3SA     | 3                   | 35      | 240   | 12.5             | 20.77      | 12.95             | 0                         | 0                                      | 16.14                        |
| H4SA     | 3                   | 35      | 240   | 12.5             | 20.23      | 13.05             | 0                         | 0                                      | 17.59                        |
| H5SA     | 3                   | 35      | 240   | 12.5             | 20.78      | 13.38             | 0                         | 0                                      | 18.23                        |
| H2SM     | 3                   | 30      | 245   | 12.5             | 20.82      | 13.19             | 0                         | 0                                      | 16.30                        |
| H3SM     | 3                   | 30      | 245   | 12.5             | 20.30      | 13.31             | 0                         | 0                                      | 18.14                        |
| H4SM     | 3                   | 30      | 245   | 12.5             | 20.76      | 12.90             | 0                         | 0                                      | 19.28                        |
| H5SM     | 3                   | 30      | 245   | 12.5             | 20.17      | 13.27             | 0                         | 0                                      | 19.74                        |
| H2SD     | 3                   | 30      | 245   | 12.0             | 20.35      | 13.70             | 0                         | 0                                      | 18.75                        |
| H3SD     | 3                   | 30      | 245   | 12.0             | 20.58      | 13.10             | 0                         | 0                                      | 20.15                        |
| H4SD     | 3                   | 30      | 245   | 12.0             | 20.37      | 13.05             | 0                         | 0                                      | 21.17                        |
| H5SD     | 3                   | 30      | 245   | 12.0             | 20.89      | 12.90             | 0                         | 0                                      | 21.71                        |
| HaSD     | 6                   | 30      | 245   | 11.5             | 20.81      | 12.58             | 0                         | 0                                      | 20.79                        |
| HaSDS10½ | 6                   | 30      | 245   | 10.5             | 20.71      | 12.06             | 0                         | 0                                      | 21.13                        |
| HaSDF    | 6                   | 30      | 245   | 11.5             | 20.52      | 12.94             | 0                         | 0                                      | 19.82                        |
| N3SO     | 3                   | 35      | 240   | 12.5             | 20.93      | 13.38             | 0                         | 0                                      | 8.95                         |
| N4SO     | 3                   | 35      | 240   | 12.5             | 20.39      | 12.42             | 0                         | 0                                      | 11.04                        |
| N5SO     | 3                   | 35      | 240   | 12.5             | 21.19      | 12.02             | 0                         | 0                                      | 13.80                        |
| N6SO     | 3                   | 35      | 240   | 12.5             | 20.46      | 13.00             | 0                         | 0                                      | 16.49                        |
| N2SA     | 3                   | 35      | 240   | 12.5             | 20.47      | 12.75             | 0                         | 0                                      | 11.12                        |
| N3SA     | 3                   | 35      | 240   | 12.5             | 20.32      | 12.31             | 0                         | 0                                      | 12.57                        |
| N4SA     | 3                   | 35      | 240   | 12.5             | 20.53      | 12.72             | 0                         | 0                                      | 14.48                        |
| N5SA     | 3                   | 35      | 240   | 12.5             | 20.92      | 12.71             | 0                         | 0                                      | 16.70                        |
| N2SD     | 6                   | 35      | 240   | 9.5              | 20.38      | 11.23             | 0                         | 1                                      | 17.60                        |
| N3SD     | 6                   | 35      | 240   | 10.0             | 21.58      | 11.22             | 0                         | 0                                      | 18.87                        |
| N4SD     | 6                   | 35      | 240   | 10.5             | 21.02      | 11.55             | 0                         | 0                                      | 19.45                        |
| N5SD     | 6                   | 35      | 240   | 10.5             | 20.37      | 11.42             | 0                         | 0                                      | 19.72                        |

TABLE 2.—(Continued)

| CODE    | BLANCH-<br>ING TIME | PROCESS |       | PUT-IN<br>WEIGHT | NET WEIGHT | DRAINED<br>WEIGHT | RUPTURED<br>OVER<br>1/16" | SINKING<br>IN 1.12<br>SP. GR.<br>BRINE | ALCOHOL-<br>INOL.<br>SOLIDS |
|---------|---------------------|---------|-------|------------------|------------|-------------------|---------------------------|--|-----------------------------|
|         |                     | TIME    | TEMP. |                  |            |                   |                           |  |                             |
|         | min.                | min.    | °F.   | ounces           | ounces     | ounces            | per cent                  | per cent                               | per cent                    |
| NaSD    | 6                   | 35      | 240   | 10.5             | 20.75      | 11.83             | 0                         | 0                                      | 19.60                       |
| NaSDF   | 6                   | 35      | 240   | 10.5             | 20.54      | 11.30             | 0                         | 0                                      | 18.29                       |
| NaSDS10 | 6                   | 35      | 240   | 10.0             | 19.66      | 11.12             | 0                         | 0                                      | 18.84                       |
| NaSDS9½ | 6                   | 35      | 240   | 9.5              | 20.58      | 10.31             | 0                         | 1                                      | 18.57                       |
| UaSB    | 4                   | 25      | 248   | 12.0             | 21.25      | 12.14             | 0                         | 0                                      | 8.18                        |
| U2SO    | 4                   | 25      | 248   | 12.0             | 21.28      | 12.60             | 0                         | 0                                      | 7.83                        |
| U3SO    | 4                   | 25      | 248   | 12.0             | 21.38      | 12.45             | 0                         | 0                                      | 9.47                        |
| U4SO    | 4                   | 25      | 248   | 12.0             | 21.41      | 12.47             | 0                         | 0                                      | 11.75                       |
| U5SO    | 4                   | 25      | 248   | 12.0             | 21.59      | 12.35             | 0                         | 0                                      | 15.10                       |
| U2SA    | 4                   | 22      | 248   | 12.0             | 21.14      | 12.23             | 0                         | 0                                      | 8.52                        |
| U3SA    | 4                   | 22      | 248   | 12.0             | 20.95      | 12.54             | 0                         | 0                                      | 10.28                       |
| U4SA    | 4                   | 22      | 248   | 12.0             | 21.49      | 12.50             | 0                         | 1                                      | 12.85                       |
| U5SA    | 4                   | 22      | 248   | 12.0             | 20.82      | 12.15             | 0                         | 0                                      | 15.62                       |
| UaSM    | 7                   | 30      | 248   | 11.0             | 21.18      | 11.73             | 0                         | 5                                      | 17.85                       |
| UaSMF   | 7                   | 30      | 248   | 11.0             | 21.30      | 11.70             | 0                         | 3                                      | 17.94                       |
| UaSMS   | 7                   | 30      | 248   | 10.0             | 21.20      | 13.80             | 0                         | 63                                     | 24.52                       |
| UaSD    | 8                   | 30      | 248   | 9.0              | 21.45      | 10.08             | 0                         | 17                                     | 22.05                       |
| UaSD/S  | 10                  | 30      | 248   | 9.0              | 21.65      | 12.88             | 0                         | 82                                     | 25.91                       |
| UaSD/F  | 8                   | 30      | 248   | 9.0              | 21.10      | 9.63              | 0                         | 12                                     | 21.54                       |
| U3HO    | 4                   | 22      | 248   | 12.0             | 21.24      | 12.52             | 0                         | 0                                      | 8.38                        |
| U5HO    | 4                   | 22      | 248   | 12.0             | 21.52      | 12.65             | 0                         | 1                                      | 12.77                       |
| UaHO    | 4                   | 22      | 248   | 12.0             | 20.94      | 12.52             | 0                         | 0                                      | 15.05                       |
| H3LB    | 3                   | 35      | 240   | 12.5             | 20.06      | 13.00             | 0                         | 0                                      | 10.15                       |
| H4LB    | 3                   | 35      | 240   | 12.5             | 20.29      | 12.91             | 0                         | 0                                      | 11.53                       |
| H5LB    | 3                   | 35      | 240   | 12.5             | 20.25      | 13.55             | 0                         | 0                                      | 14.27                       |
| HaLO    | 3                   | 35      | 240   | 12.5             | 20.48      | 13.62             | 0                         | 0                                      | 15.97                       |
| HaLa    | 3                   | 35      | 240   | 12.5             | 20.81      | 13.38             | 0                         | 0                                      | 18.45                       |
| HaLM    | 3                   | 30      | 245   | 12.5             | 20.71      | 13.50             | 0                         | 0                                      | 21.07                       |
| HaLD    | 3                   | 30      | 245   | 12.5             | 20.30      | 13.59             | 0                         | 0                                      | 20.40                       |
| HaLDS11 | 6                   | 30      | 245   | 11.0             | 20.75      | 13.08             | 0                         | 0                                      | 20.82                       |
| HaLDF   | 6                   | 30      | 245   | 11.5             | 20.45      | 13.38             | 0                         | 0                                      | 19.35                       |
| D6GO    | 4                   | 40      | 242   | 12.0             | 21.05      | 12.03             | 0                         | 0                                      | 11.72                       |

which practically all the vines and pods were dry and yellow, but the shelled peas were still soft and succulent, although not so desirable as those obtained in the earlier series.

Some of the packs have a fifth code letter, F or S, F designating those that floated in brine, and S those that sank. Numerals following these letters refer to the put-in weight of the blanched peas. Some canners attempt to separate the more mature peas which may occur in the run-of-pod peas as they come from the viner by means of brine flotation. The

cannery in Utah uses such a brine separator, the brine being kept at a specific gravity of 1.07 to 1.10, according to the judgment of the superintendent. Wherever possible these experimental packs were run through this brine separator. No brine separator was available at the other canneries, but for experimental purposes certain packs were made in which the raw peas were separated by means of a brine of a specific gravity similar to that used in the Utah plant. As will be seen from the tables, the peas that sank in the brine were usually of inferior quality to those that floated, but there are indications that brine separation is most useful when the majority of peas are very young. In such instances the brine separator prevents any occasional more mature peas being mixed with the young peas and results in a higher quality pack. If there are a considerable number of mature, or nearly mature, peas present, the use of a brine separator is not profitable, because the floaters are not much better than the sinkers.

With the "I" packs three special marks appear, "WB" indicating that the factory brine, rather than special brine made from softened water, was used, "12" meaning that the peas were blanched 12 minutes instead of 4; and "6H" indicating that the raw peas were held for 6 hours before blanching and canning. These peas constitute special packs, which were included to permit an opportunity to compare special conditions with the regular experimental packs.

Results of the examination of the samples are given in Table 1, Alaska peas, and Table 2, sweet peas. As will be noted, each sample is designated by a code, the meaning of which is described above. For example, MaAD/S11 means that the can was packed in Walkerton, Va., from run-of-pod Alaska peas, which were distinctly mature. The peas were sinkers from a brine separation, and 11 ounces of blanched peas were present in the can.

The following methods were used in making the determinations indicated in the tables:

#### DRAINED WEIGHT

Transfer the contents of a can to a dish and mix with a volume of tap water equal to twice the volume of the sample. Pour the mixture on an 8-mesh screen, 8 inches in diameter, in such a manner as to spread the peas evenly. Tilt the screen as much as possible without shifting the peas and drain for 2 minutes. Remove surplus moisture from the lower surface of the screen with a cloth, pour the drained peas into a tared dish, and determine the weight.

#### PER CENT RUPTURED OVER 1/16 INCH

In a sample of 100 of the drained peas, count the number that show the skin to have been broken open, due to internal pressure, to such an extent as to separate the broken edges more than 1/16 inch. Do not consider as ruptured the peas that show evidence of having been broken by crushing or other external pressure.

#### PER CENT SINKING IN 1.12 SPECIFIC GRAVITY BRINE

Pour a weighed amount of drained peas into a brine of 1.12 specific gravity at 65°F. Use a brine container of such size that the brine is a little over 4 inches deep



and the floating peas form a single layer (for a No. 2 can a rectangular dish, 4½ inches deep, 5 inches wide and 10 inches long, was found convenient). After 15 seconds skim off the floating peas, wash, drain and weigh as above. Determine per cent sinking in brine by difference.

#### ALCOHOL-INSOLUBLE SOLIDS<sup>1</sup>

Grind a sample of drained peas in a food chopper, stir until homogeneous and weigh 20 grams into a 600 cc. beaker. Add 300 cc. of alcohol, 80 per cent by volume, with stirring. Cover the beaker and bring mixture to a boil. After simmering slowly for 30 minutes filter with suction on a tared dry filter paper fitted into a Büchner funnel. Wash the material on the filter with 80 cc. alcohol until washings are clear and colorless. Transfer the filter paper and solids to the covered dish originally used in drying and taring the filter paper, dry with the dish uncovered for 2 hours at the temperature of boiling water, place cover on dish, cool in a desiccator, and weigh.

TABLE 3.—*Correlation between maturity of the field and alcohol-insoluble solids*

| ALCOHOL-INSOLUBLE<br>SOLIDS—PER CENT | MATURITY     |   |   |    |    |   |    |    |    |    |
|--------------------------------------|--------------|---|---|----|----|---|----|----|----|----|
|                                      | B            |   | O |    | A  |   | M  |    | D  |    |
|                                      | ALASKA SWEET |   | A |    | S. |   | A. |    | S. |    |
|                                      |              |   |   |    |    |   |    |    |    |    |
| per cent                             |              |   |   |    |    |   |    |    |    |    |
| 5 — 7.5                              | 2            | 1 | 0 | 1  | 0  | 1 | 0  | 0  | 0  | 0  |
| 7.51–10                              | 7            | 7 | 3 | 9  | 2  | 3 | 0  | 1  | 0  | 0  |
| 10.01–12.5                           | 9            | 7 | 4 | 14 | 4  | 5 | 2  | 3  | 0  | 0  |
| 12.51–15                             | 8            | 5 | 2 | 8  | 1  | 8 | 0  | 1  | 0  | 0  |
| 15.01–17.5                           | 3            | 0 | 7 | 5  | 6  | 8 | 1  | 7  | 1  | 1  |
| 17.51–20                             | 1            | 0 | 3 | 0  | 5  | 5 | 2  | 10 | 0  | 11 |
| 20.01–22.5                           | 0            | 0 | 0 | 0  | 7  | 0 | 3  | 2  | 1  | 12 |
| 22.51–25                             | 0            | 0 | 0 | 0  | 0  | 0 | 14 | 0  | 6  | 1  |
| 25.01–27.5                           | 0            | 0 | 0 | 0  | 0  | 0 | 5  | 0  | 17 | 0  |
| 27.51–30                             | 0            | 0 | 0 | 0  | 0  | 0 | 0  | 0  | 1  | 0  |

TABLE 4.—*Correlation between maturity and per cent sinking*

| PEAS SINKING IN<br>1.12 BRINE | MATURITY |    |    |   |   |
|-------------------------------|----------|----|----|---|---|
|                               | B        | O  | A  | M | D |
| <i>per cent</i>               |          |    |    |   |   |
| 0-10                          | 29       | 19 | 22 | 3 | 1 |
| 11-20                         | 0        | 0  | 3  | 4 | 0 |
| 21-30                         | 0        | 0  | 0  | 2 | 2 |
| 31-40                         | 0        | 0  | 0  | 4 | 2 |
| 41-50                         | 0        | 0  | 0  | 5 | 1 |
| 51-60                         | 0        | 0  | 0  | 5 | 3 |
| 61-70                         | 0        | 0  | 0  | 1 | 4 |
| 71-80                         | 0        | 0  | 0  | 0 | 5 |
| 81-90                         | 0        | 0  | 0  | 0 | 2 |
| 91-100                        | 0        | 0  | 0  | 0 | 5 |

<sup>1</sup> Modification of method proposed by Z. I. Kertess, *Food Ind.*, 6, 168 (1934).

Table 3 illustrates the correlation between maturity of the field and alcohol-insoluble solids. For example, two samples of Alaska peas, of the maturity coded as B, showed alcohol-insoluble solids between 5 and 7.5 per cent.

Table 4 illustrates with Alaska peas the correlation between maturity and per cent sinking in a brine of 1.12 specific gravity.

Table 5 illustrates with Alaska peas the correlation between maturity and per cent peas so swollen as to rupture the skin more than 1/16".

TABLE 5.—*Correlation between maturity and per cent swollen excessively*

| PEAS RUPTURED<br>OVER 1/16 INCH | MATURITY |    |    |    |   |
|---------------------------------|----------|----|----|----|---|
|                                 | B        | O  | A  | M  | D |
| <i>per cent</i>                 |          |    |    |    |   |
| 0-10                            | 29       | 19 | 25 | 19 | 9 |
| 11-20                           | 0        | 0  | 0  | 3  | 2 |
| 21-30                           | 0        | 0  | 0  | 2  | 2 |
| 31-40                           | 0        | 0  | 0  | 0  | 4 |
| 41-50                           | 0        | 0  | 0  | 1  | 2 |
| 51-60                           | 0        | 0  | 0  | 0  | 5 |
| 61-70                           | 0        | 0  | 0  | 0  | 2 |

#### SUMMARY

Analyses of canned peas from eleven states, consisting of 157 samples of Alaska peas and 159 samples, including 8 varieties, of sweet peas, showed alcohol-insoluble solids to be an excellent index of maturity for both Alaska and sweet peas. With the exception of one sample, Minn4AM, which showed alcohol-insoluble solids of 22.8 per cent, alcohol-insoluble solids were below 22 per cent in all samples of Alaska peas which were not mature, or which were not quite mature. The corresponding figure for sweet peas was 19 per cent.

With Alaska peas no sample which was not packed from mature peas was found with over 5 per cent peas which were so swollen as to rupture the skin more than 1/16 inch. Similarly, no peas which were not packed from mature peas were found which showed over 17 per cent which sank in a brine of 1.12 specific gravity. It should be noted that all these studies were made on peas packed in brine of practically identical composition. No study was made of the effect of varying packing mediums on the specific gravity of the peas. It was found that sweet peas, even though mature, do not rupture appreciably when canned, and that their specific gravity is enough below that of Alaska peas so that in very few instances, even when mature peas are canned, do any appreciable number sink in a brine of 1.12 specific gravity.

## A STUDY OF CHEMICAL AND PHYSICAL METHODS FOR DETERMINING THE MATURITY OF CANNED SNAP (STRINGLESS) BEANS

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This investigation was undertaken during the season of 1935 to obtain data that may be useful in establishing a minimum standard of quality for canned snap (stringless) beans, as provided in the McNary-Mapes amendment to the Federal Food and Drugs Act. The 104 samples used represented seven different varieties from seven different states throughout the country.

Beans to be used in making the various packs were obtained from fields selected by the factory superintendents and field men to show various stages of maturity. The opinion of these men was obtained in regard to what commercial grade of canned product each lot of beans at a particular stage of maturity would make. The stages of maturity were designated as "Tender," "Fairly Tender," and "Distinctly Mature." The "Tender" classification corresponded to the commercial grade "Fancy," or "Extra Standard," while the "Fairly Tender" and "Distinctly Mature" corresponded to the commercial grades "Standard" and "Substandard," respectively. These terms may be defined as follows:

*Tender*.—Small or medium sized pods that snapped readily when bent. The seeds were undeveloped in the pod.

*Fairly Tender*.—Large pods that still snapped when bent. The seeds were well developed but tender.

*Distinctly Mature*.—Large pods, tough, leathery, rubbery, or limp, that would no longer snap when bent. The seeds were generally well developed and slightly shriveled.

After the beans had been picked and sorted into the various maturities described, and the ends had been snipped off by hand, they were cut into lengths of about one inch. They were then blanched in boiling water. All cans were filled by hand as full as possible, the interstices being filled with the factory brine, usually 2–3 per cent salt.

A preliminary investigation indicated that the amount of tough, fibrous material in the pods and the percentage of seeds present would be of value in determining the maturity of the beans. Accordingly, these determinations, as well as the determination of alcohol-insoluble solids, were made on all samples of the canned product. In addition, each sample was graded by six expert canned food graders, the grade numbers 1 (fancy), 2 (extra standard), 3 (standard), and 4 (substandard) being assigned to each sample, respectively. Frequently the grader would assign a plus or minus value to the grade. In calculating the average grade for

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<sup>1</sup> W. B. White, Chief.

such a sample, three-tenths (.3) was added for a plus value and subtracted for a minus value. For example, a sample given a rating of 3+ was assigned the score of 3.3, and a sample given a rating of 3- was assigned a score of 2.7. The chemical determinations were made by the following procedures.

#### METHODS OF ANALYSIS

(1) *Drained Weight of Beans*.—Transfer the contents of the can to a container. Add 2 cans of water, mix, and spread the material on an 8-mesh screen, using an 8-inch screen for containers of less than 3 pounds net weight, and a 12-inch screen for larger containers. Tilt the screen as much as possible without shifting the beans and drain for 2 minutes, wiping with a cloth the excess moisture from the lower surface of the screen. Transfer the beans to a tared dish and weigh.

(2) *Seeds*.—Weigh 150 grams of the material drained as directed under (1), and separate and weigh the seeds.

(3) *Fibrous Material*.—Cut 100 grams of the pods, separated from the seeds as directed under (2), into pieces approximately  $\frac{1}{4}$  inch in length. Pulp the sample in a large mortar, transfer to the metal cup of a malted milk mixer with 200 cc. of boiling water, and add a small piece of paraffin. Bring the mixture to a boil and add 25 cc. of 50 per cent sodium hydroxide solution. After boiling exactly 5 minutes, stir for exactly 5 minutes with a malted milk stirrer (capable of a no-load speed of at least 7200 r.p.m.). Filter, with suction, through a tared 30-mesh monel metal screen fitted into a Büchner funnel, washing the pulp through the screen with a  $\frac{1}{4}$  inch stream of boiling water. After washing free of alkalinity (1–1.5 liters of water), further wash the fiber on the screen with a stream of boiling water until the pulp is removed and the washings are clear. Dry the screen and fiber for 2 hours at 100°C., cool, and weigh.

(4) *Alcohol-Insoluble Solids*.—Treat the remainder of the drained material as directed for canned peas, *This Journal*, 19, 618 (1936).

The varieties and sources of the beans, together with the results of the examinations and other information, are given in Table 1. The column headed "Pods" represents the condition of the pods before canning. The word "commercial" used in connection with the sample means a regular commercial pack sampled from the factory warehouse.

Three charts were also prepared to show the correlation between the various constituents of the bean and the grade of the product. Chart 1 shows the division of the samples with respect to fibrous material and grade. It will be noted that 0.08 per cent fibrous material is the maximum limit for Grade 3 beans. Beyond this point one or more of the graders called the product Grade 4. Similarly, Chart 2 shows the relationship of seed percentage to grade. With a single exception the maximum figure for a Grade 3 product is 6 per cent. Chart 3 shows a division of the various samples with respect to alcohol-insoluble solids and grade. Here the maximum figure for a Grade 3 product is 7 per cent. The percentage of alcohol-insoluble solids does not appear to be as diagnostic as the percentage of fibrous material and the proportion of seeds to pod. This is apparent from the fact that 32 samples grading more than No. 3 were regarded as satisfactory as judged by the maximum figure of 7 per cent for alcohol-in-

TABLE I

| INV. NO. | SOURCE       | STYLE     | PODS                   | BLANCH<br>TIME | TEMP.       | COOK<br>TIME | TEMP. | ALCOHOL-<br>INSOLUBLE<br>SOLIDS | FIBROUS<br>MATERIAL<br>IN PODS | SEEDS    | GRADE |
|----------|--------------|-----------|------------------------|----------------|-------------|--------------|-------|---------------------------------|--------------------------------|----------|-------|
|          |              |           |                        | min.           | °F.         | min.         | °F.   | per cent                        | per cent                       | per cent |       |
| 13195B a | * California | Cut       | Tender                 | 2              | 195         | 22           | 240   | 5.03                            | 0.010                          | 1.5      | 1.0   |
| " b      | "            | "         | Fairly tender          | 2              | 195         | 22           | 240   | 4.26                            | 0.062                          | 5.0      | 2.4   |
| " c      | "            | "         | Distinctly mature      | 2              | 195         | 22           | 240   | 5.66                            | 0.260                          | 10.0     | 4.0   |
| 13196B a | "            | " #1      | Commercial             | 2              | 195         | 22           | 240   | 4.94                            | 0.013                          | 1.5      | 1.1   |
| " b      | "            | " #2      | "                      | 2              | 195         | 22           | 240   | 4.16                            | 0.026                          | 2.0      | 1.7   |
| " c      | "            | " #4      | "                      | 2              | 195         | 22           | 240   | 4.01                            | 0.038                          | 4.0      | 2.5   |
| " d      | "            | " #5      | "                      | 2              | 195         | 22           | 240   | 4.10                            | 0.146                          | 5.2      | 3.6   |
| 40351B a | Oregon       | " #5      | Distinctly mature      | 5              | Boil. water | 25           | 240   | 4.14                            | 0.221                          | 6.5      | 3.6   |
| " b      | "            | "         | Fairly tender          | 5              | "           | 25           | 240   | 4.37                            | 0.102                          | 4.0      | 3.3   |
| " c      | "            | " #2      | Tender                 | 5              | "           | 25           | 240   | 4.14                            | 0.032                          | 2.0      | 1.7   |
| " d      | "            | " #5      | Distinctly mature      | None           | None        | 25           | 240   | 4.25                            | 0.176                          | 6.0      | 3.9   |
| 40352B   |              | " #5      | "                      | 4              | Steam       | 20           | 240   | 5.64                            | 0.542                          | 21.5     | 4.1   |
| 40353B a | Washington   | Cut       | Commercial substandard | 4              | "           | 20           | 240   | 5.80                            | 0.279                          | 4.5      | 4.1   |
| " b      | "            | "         | Commercial standard    | 4              | "           | 20           | 240   | 4.41                            | 0.083                          | 4.0      | 3.5   |
| " c      | "            | "         | Commercial choice      | 4              | "           | 20           | 240   | 4.41                            | 0.019                          | 1.5      | 2.6   |
| " d      | "            | " #4      | "                      | 1-2            | 160-200     | 27           | 240   | 4.45                            | 0.042                          | 4.5      | 2.2   |
| 40361B a | "            | Whole #1  | Commercial fancy       | 1-2            | 160-200     | 27           | 240   | 4.79                            | 0.009                          | Trace    | 1.0   |
| " b      | "            | " #6      | Distinctly mature      | 1-2            | 160-200     | 27           | 240   | 5.83                            | 0.215                          | 10.5     | 3.6   |
| 40364B a | "            | Whole     | "                      | 4†             | 190         | 22           | 248   | 4.92                            | 0.134                          | 10.0     | 4.0   |
| " b      | "            | "         | Commercial fancy       | 22             | 248         | 22           | 248   | 3.57                            | 0.034                          | 4.0      | 2.2   |
| " c      | "            | Whole #1  | "                      | 22             | 248         | 22           | 248   | 4.11                            | 0.014                          | Trace    | 1.0   |
| " d      | "            | Cut #5    | Commercial standard    | 22             | 248         | 22           | 248   | 3.39                            | 0.038                          | 5.0      | 3.3   |
| 46102B a | Oregon       | Asparagus | Commercial fancy       | 1†             | 185         | 20           | 242   | 3.04                            | 0.020                          | 3.5      | 2.3   |
| " b      | "            | "         | Commercial standard    | 2              | 185         | 20           | 242   | 4.36                            | 0.078                          | 7.0      | 3.9   |
| " c      | "            | "         | Commercial substandard | 2              | 185         | 30           | 242   | 4.53                            | 0.166                          | 8.0      | 4.0   |
| 39457B   | † Michigan   | "         | Distinctly mature      | 4              | 166         | 20           | 240   | 12.75                           | 0.144                          | 27.5     | 4.0   |
| 39458B   | "            | "         | Fairly tender          | 4              | 166         | 20           | 240   | 6.31                            | 0.027                          | 14.5     | 3.0   |
| 39459B   | "            | "         | Small—tender           | 4              | 166         | 20           | 240   | 3.91                            | 0.023                          | 3.0      | 1.6   |
| 39463B   | "            | "         | Distinctly mature      | 4              | 160         | 20           | 240   | 14.37                           | 0.146                          | 39.0     | 4.2   |
| 39464B   | "            | "         | Fairly tender          | 4              | 160         | 20           | 240   | 6.73                            | 0.046                          | 14.5     | 3.4   |
| 39465B   | "            | "         | Small—tender           | 4              | 160         | 20           | 240   | 4.17                            | 0.020                          | 6.0      | 2.1   |
| NVG S    | New Jersey   | "         | Distinctly mature      | 10             | 212         | 20           | 240   | 10.27                           | 0.122                          | 26.0     | 3.9   |
| NJG S    | Delaware     | "         | "                      | 5              | 212         | 25           | 240   | 9.07                            | 0.098                          | 23.5     | 4.0   |
| FRG S    | New Jersey   | "         | Commercial             | 3†             | 206         | 20           | 240   | 4.99                            | 0.035                          | 6.5      | 3.2   |
| NJG P    | "            | "         | Distinctly mature      | 10             | 212         | 20           | 240   | 11.82                           | 0.430                          | 29.5     | 4.1   |

TABLE 1.—(Continued)

| INV. NO. | SOURCE     | STYLE             | PODS                | BLANCH |         | COOK | ALCOHOL-<br>INSOLUBLE |     | FIBROUS<br>MATERIAL |          | SEEDS | GRADE |
|----------|------------|-------------------|---------------------|--------|---------|------|-----------------------|-----|---------------------|----------|-------|-------|
|          |            |                   |                     | min.   | °F.     |      | min.                  | °F. | per cent            | per cent |       |       |
| 40355B   | Oregon     | "                 | Distinctly mature   | None   |         | 25   | 25                    | 240 | 5.27                | 0.068    | 8.0   | 3.8   |
| 40357B a |            | "                 | Fairly tender       | "      |         | 25   | 25                    | 240 | 4.25                | 0.043    | 8.0   | 3.1   |
| 40357B b | Washington | Whole             | Tender              | "      |         | 20   | 20                    | 240 | 3.72                | 0.058    | 2.5   | 2.1   |
| 40360B a |            | "                 | Distinctly mature   | 3½     | 200     | 20   | 20                    | 240 | 7.31                | 0.122    | 19.0  | 4.1   |
| 40360B b |            | " #2              | Commercial fancy    | 3½     | 200     | 20   | 20                    | 240 | 4.66                | 0.039    | 3.5   | 1.8   |
| 40362-Ba |            | " #6              | Commercial standard | 3½     | 200     | 20   | 20                    | 240 | 4.67                | 0.057    | 4.0   | 3.0   |
| 40362-Bb | "          | Cut #5            | Commercial standard | 1-2    | 180-200 | 27   | 240                   | 240 | 4.57                | 0.092    | 7.0   | 4.0   |
| 40362-Bc |            | " #6              | Distinctly mature   | 1-2    | 180-200 | 27   | 240                   | 240 | 4.49                | 0.082    | 4.0   | 2.2   |
| 40362-Bd |            | " #4              | Commercial choice   | 1-2    | 180-200 | 27   | 240                   | 240 | 4.99                | 0.032    | 3.0   | 2.0   |
| 40363B a |            | Whole #1, #2      | Commercial fancy    | 1-2    | 180-200 | 27   | 240                   | 240 | 4.65                | 0.033    | 3.0   | 1.8   |
| 40363B b | Oregon     | Whole             | "                   | 1½     | 160-180 | 30   | 240                   | 240 | 4.66                | 0.076    | 3.5   | 2.7   |
| 40363B c |            | "                 | Commercial standard | 1½     | 160-180 | 30   | 240                   | 240 | 4.56                | 0.042    | 3.0   | 1.5   |
| 40363B d |            | "                 | Commercial fancy    | 2-3    | 180-200 | 25   | 240                   | 240 | 4.56                | 0.042    | 3.0   | 1.5   |
| 40363B e |            | "                 | Commercial standard | 2-3    | 180-200 | 25   | 240                   | 240 | 4.09                | 0.043    | 4.5   | 3.1   |
| 40363B f | California | Whole             | Distinctly mature   | 2-3    | 180-200 | 25   | 240                   | 240 | 4.50                | 0.083    | 11.0  | 3.6   |
| 40363B g |            | Asparagus #3      | Tender              | 3½     | 175-180 | 27   | 238                   | 238 | 4.55                | 0.033    | 3.0   | 1.4   |
| 40363B h |            | " #5              | Fairly tender       | 3½     | 175-180 | 27   | 238                   | 238 | 4.69                | 0.023    | 4.5   | 2.8   |
| 40363B i |            | " #6              | Distinctly mature   | 3½     | 175-180 | 27   | 238                   | 238 | 4.48                | 0.045    | 5.0   | 3.3   |
| 40363B j | "          | Cut #2            | Tender              | 3½     | 175-180 | 27   | 238                   | 238 | 4.11                | 0.025    | 2.0   | 1.2   |
| 40363B k |            | " #3              | "                   | 3½     | 175-180 | 27   | 238                   | 238 | 4.08                | 0.037    | 3.0   | 2.4   |
| 40363B l |            | " #5              | Fairly tender       | 3½     | 175-180 | 27   | 238                   | 238 | 4.54                | 0.053    | 3.5   | 3.3   |
| 40363B m |            | " #7              | Distinctly mature   | 3½     | 175-180 | 27   | 238                   | 238 | 4.78                | 0.044    | 4.5   | 3.5   |
| 40363B n | "          | Whole #1          | "                   | 3½     | 175-180 | 27   | 238                   | 238 | 4.98                | 0.081    | 7.3   | 4.1   |
| 40363B o |            | Cut #2            | Tender              | 3      | 196-198 | 22   | 240                   | 240 | 4.44                | 0.022    | 1.5   | 1.6   |
| 40363B p |            | " #3              | "                   | 3      | 196-198 | 22   | 240                   | 240 | 4.53                | 0.028    | 1.0   | 1.0   |
| 40363B q |            | " #5, #6, #7      | Distinctly mature   | 3      | 196-198 | 22   | 240                   | 240 | 4.46                | 0.039    | 2.5   | 2.5   |
| 40363B r | "          | Asparagus #2      | Tender              | 3      | 196-198 | 22   | 240                   | 240 | 5.54                | 0.094    | 8.5   | 3.9   |
| 40363B s |            | " #3              | "                   | 3      | 196-198 | 22   | 240                   | 240 | 4.85                | 0.017    | 3.5   | 1.4   |
| 40363B t |            | " #4              | "                   | 3      | 196-198 | 22   | 240                   | 240 | 4.53                | 0.025    | 3.0   | 2.0   |
| 40363B u |            | " #5              | Distinctly mature   | 3      | 196-198 | 22   | 240                   | 240 | 4.68                | 0.011    | 5.0   | 3.0   |
| 40363B v | "          | Cut #2            | Tender              | 3      | 196-198 | 22   | 240                   | 240 | 5.00                | 0.088    | 7.0   | 3.5   |
| 40363B w |            | " #5, #6, #7      | "                   | 2      | 195     | 22   | 240                   | 240 | 4.55                | 0.028    | 1.5   | 2.0   |
| 40363B x |            | " #4              | "                   | 2      | 195     | 22   | 240                   | 240 | 4.51                | 0.047    | 3.0   | 2.7   |
| 40363B y |            | " #5              | Fairly tender       | 2      | 195     | 22   | 240                   | 240 | 5.32                | 0.070    | 5.5   | 3.3   |
| 40363B z | Michigan   | " #6              | Distinctly mature   | 2      | 195     | 22   | 240                   | 240 | 7.19                | 0.335    | 9.5   | 4.0   |
| 39451B   |            | Cut               | Distinctly mature   | 4      | 200-212 | 22   | 240                   | 240 | 12.20               | 0.739    | 34.5  | 3.8   |
| 39455B   |            | Fairly tender     | "                   | 4      | 200-212 | 22   | 240                   | 240 | 3.89                | 0.063    | 5.5   | 2.8   |
| 39456B   |            | Tender            | "                   | 4      | 200-212 | 22   | 240                   | 240 | 3.48                | 0.036    | 2.5   | 1.1   |
| 39460B   | "          | Distinctly mature | "                   | 4      | 160     | 20   | 240                   | 240 | 10.55               | 0.708    | 34.2  | 3.9   |
| 39461B   |            | Fairly tender     | "                   | 4      | 160     | 20   | 240                   | 240 | 4.33                | 0.051    | 7.3   | 3.3   |
| 39462B   |            | Tender            | "                   | 4      | 160     | 20   | 240                   | 240 | 3.69                | 0.035    | 3.0   | 1.6   |

TABLE 1.—(Continued)

| INT. NO.      | SOURCE        | STYLE | PODS                | BLANCH |             | COOK | ALCOHOL-<br>INSOLUBLE |          | FIBROUS<br>MATERIAL | SEEDS    | GRADE |
|---------------|---------------|-------|---------------------|--------|-------------|------|-----------------------|----------|---------------------|----------|-------|
|               |               |       |                     | TIME   | TEMP.       | TIME | ° F.                  | per cent |                     | per cent |       |
| 40354B        | †† Oregon     | "     | Distinctly mature   | min.   | ° F.        | min. | ° F.                  | per cent | per cent            | per cent |       |
| 40356B a      |               | "     | Tender              | 5      | None        | 25   | 200                   | 15.46    | 0.312               | 32.0     | 4.0   |
| " b           |               | "     | Fairly tender       | 5      | "           | 20   | 240                   | 4.38     | 0.022               | 5.0      | 2.5   |
| 46103B a      |               | " #6  | Commercial          | 5      | "           | 20   | 240                   | 5.23     | 0.023               | 3.2      | 3.1   |
| " b           | " #6          | "     | "                   |        |             |      |                       | 4.86     | 0.028               | 4.0      | 2.8   |
| " c           |               | "     | "                   |        |             |      |                       | 4.37     | 0.020               | 4.5      | 2.6   |
| " d           |               | " #2  | "                   |        |             |      |                       | 4.15     | 0.020               | 2.0      | 1.7   |
| N.Y. Wax a    | ** New York   | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 10.59    | 0.086               | 27.5     | 3.8   |
| " b           |               | "     | Fairly tender       | 5      | 200         | 20   | 240                   | 5.40     | 0.018               | 6.0      | 2.8   |
| " c           |               | "     | Tender              | 5      | 200         | 20   | 240                   | 4.62     | 0.018               | 4.0      | 2.0   |
| " d           |               | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 7.35     | 0.457               | 30.5     | 4.0   |
| N.Y. Flat Wax | "             | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 9.75     | 9.516               | 22.0     | 4.0   |
| 39452B        |               | "     | Distinctly mature   | 4      | 200         | 22   | 240                   | 10.42    | 0.223               | 30.5     | 3.9   |
| 39453B        |               | "     | Fairly tender       | 4      | 200         | 22   | 240                   | 7.79     | 0.135               | 18.5     | 3.9   |
| 39454B        |               | "     | Tender              | 4      | 200         | 22   | 240                   | 3.59     | 0.063               | 3.0      | 1.5   |
| 40350B a      | Oregon        | "     | Distinctly mature   | 3      | Boil. water | 20   | 240                   | 7.02     | 0.124               | 15.0     | 3.8   |
| " b           |               | Whole | Commercial fancy    | 2      | "           | 20   | 240                   | 4.03     | 0.013               | 1.5      | 1.4   |
| " c           |               | Cut   | Commercial standard | 2      | "           | 20   | 240                   | 5.25     | 0.052               | 6.0      | 3.0   |
| N Y H a       | New York      | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 10.78    | 0.433               | 29.5     | 4.0   |
| " b           |               | "     | Fairly tender       | 5      | 200         | 20   | 240                   | 7.26     | 0.117               | 17.0     | 3.9   |
| " c           |               | "     | Tender              | 5      | 200         | 20   | 240                   | 4.39     | 0.036               | 6.0      | 2.5   |
| " d           |               | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 10.81    | 0.340               | 30.0     | 3.9   |
| " e           |               | "     | Commercial          | 5      | 200         | 20   | 240                   | 5.71     | 0.121               | 14.0     | 3.4   |
| N Y F a       | "             | "     | Distinctly mature   | 5      | 200         | 20   | 240                   | 9.85     | 0.412               | 28.5     | 4.1   |
| " b           |               | "     | Fairly tender       | 5      | 200         | 20   | 240                   | 6.18     | 0.104               | 10.5     | 3.6   |
| " c           |               | "     | Tender              | 5      | 200         | 20   | 240                   | 4.99     | 0.034               | 5.5      | 2.4   |
| B S           | §§ New Jersey | "     | Commercial standard | 3      | 212         | 20   | 240                   | 4.05     | 0.022               | 2.5      | 2.3   |
| F R B S       |               | "     | "                   | 3      | 206         | 20   | 240                   | 4.91     | 0.031               | 5.5      | 3.4   |
| B S Dela      |               | "     | Distinctly mature   | 5      | 200         | 25   | 240                   | 5.05     | 0.018               | 6.0      | 3.4   |
| " b           | Delaware      | "     | "                   | 5      | 200         | 25   | 240                   | 9.35     | 0.090               | 20.0     | 4.1   |

\* Blue Lake.

† Giant Stringless.

† Stringless Green Pod.

§ Kentucky Wonder.

†† Refuge.

§§ Burpee's Stringless.

†† Wax.

\*\* Improved Kidney Wax.

soluble solids, whereas in the case of the maximum figures for fiber and seeds, only 15 samples would be regarded as satisfactory in each case.

CHART 1

|                           |          |       |         |         |         |         |         |         |
|---------------------------|----------|-------|---------|---------|---------|---------|---------|---------|
| PER CENT FIBROUS MATERIAL | .761-.80 |       |         |         |         |         |         |         |
|                           | .721-.76 |       |         |         |         |         | 1       |         |
|                           | .681-.72 |       |         |         |         |         | 1       |         |
|                           | .641-.68 |       |         |         |         |         |         |         |
|                           | .601-.64 |       |         |         |         |         |         |         |
|                           | .561-.60 |       |         |         |         |         |         |         |
|                           | .521-.56 |       |         |         |         |         |         | 1       |
|                           | .481-.52 |       |         |         |         |         | 1       |         |
|                           | .441-.48 |       |         |         |         |         | 1       |         |
|                           | .401-.44 |       |         |         |         |         | 1       | 2       |
|                           | .361-.40 |       |         |         |         |         |         |         |
|                           | .321-.36 |       |         |         |         |         | 2       |         |
|                           | .281-.32 |       |         |         |         |         | 1       |         |
|                           | .241-.28 |       |         |         |         |         | 1       | 1       |
|                           | .201-.24 |       |         |         |         |         | 3       |         |
|                           | .161-.20 |       |         |         |         |         | 2       |         |
|                           | .121-.16 |       |         |         |         | 1       | 6       | 2       |
|                           | .081-.12 |       |         |         |         | 2       | 7       | 2       |
|                           | .041-.08 | 2     |         | 4       | 5       | 9       | 1       |         |
|                           | 0-.04    | 11    | 12      | 10      | 7       | 5       |         |         |
|                           |          | 1-1.5 | 1.6-2.0 | 2.1-2.5 | 2.6-3.0 | 3.1-3.5 | 3.6-4.0 | 4.1-4.5 |
| GRADE                     |          |       |         |         |         |         |         |         |

Many of the samples that were regarded as substandard by the expert graders and found to be satisfactory by the chemical and physical meth-



CHART 2

|                |         |       |         |         |         |         |         |         |
|----------------|---------|-------|---------|---------|---------|---------|---------|---------|
| PER CENT SEEDS | 38.1-40 |       |         |         |         |         |         | 1       |
|                | 36.1-38 |       |         |         |         |         |         |         |
|                | 34.1-36 |       |         |         |         |         | 2       |         |
|                | 32.1-34 |       |         |         |         |         | 1       |         |
|                | 30.1-32 |       |         |         |         |         | 2       |         |
|                | 28.1-30 |       |         |         |         |         | 2       | 2       |
|                | 26.1-28 |       |         |         |         |         | 2       |         |
|                | 24.1-26 |       |         |         |         |         | 1       |         |
|                | 22.1-24 |       |         |         |         |         | 1       |         |
|                | 20.1-22 |       |         |         |         |         | 1       | 1       |
|                | 18.1-20 |       |         |         |         |         | 1       | 2       |
|                | 16.1-18 |       |         |         |         |         | 1       |         |
|                | 14.1-16 |       |         |         | 1       | 1       | 1       |         |
|                | 12.1-14 |       |         |         |         | 1       |         |         |
|                | 10.1-12 |       |         |         |         |         | 3       |         |
|                | 8.1-10  |       |         |         |         |         | 4       |         |
|                | 6.1-8   |       |         |         |         | 3       | 4       | 2       |
|                | 4.1-6   |       |         | 6       | 6       | 7       | 2       | 1       |
|                | 2.1-4   | 5     | 7       | 8       | 4       | 5       |         |         |
|                | 0-2.0   | 7     | 5       |         | 1       |         |         |         |
|                |         | 1-1.5 | 1.6-2.0 | 2.1-2.5 | 2.6-3.0 | 3.1-3.5 | 3.6-4.0 | 4.1-4.5 |
| GRADE          |         |       |         |         |         |         |         |         |

ods used, contained an excessive amount of strings, particularly in the Kentucky Wonder variety. There was a decidedly objectionable string development, but the pods did not contain sufficient fibrous material to render the product unsatisfactory when compared with other varieties.

CHART 3

|                                   |         |         |         |         |         |         |         |         |
|-----------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| PER CENT ALCOHOL-INSOLUBLE SOLIDS | 19.1-20 |         |         |         |         |         |         |         |
|                                   | 18.1-19 |         |         |         |         |         |         |         |
|                                   | 17.1-18 |         |         |         |         |         |         |         |
|                                   | 16.1-17 |         |         |         |         |         |         |         |
|                                   | 15.1-16 |         |         |         |         |         | 1       |         |
|                                   | 14.1-15 |         |         |         |         |         |         | 1       |
|                                   | 13.1-14 |         |         |         |         |         |         |         |
|                                   | 12.1-13 |         |         |         |         |         | 2       |         |
|                                   | 11.1-12 |         |         |         |         |         |         | 1       |
|                                   | 10.1-11 |         |         |         |         |         | 6       |         |
|                                   | 9.1-10  |         |         |         |         |         | 3       | 2       |
|                                   | 8.1- 9  |         |         |         |         |         |         | 1       |
|                                   | 7.1- 8  |         |         |         |         |         | 4       | 1       |
|                                   | 6.1- 7  |         |         |         | 1       | 2       | 1       |         |
|                                   | 5.1- 6  | 1       |         |         | 2       | 4       | 4       | 1       |
|                                   | 4.1- 5  | 9       | 9       | 11      | 7       | 10      | 8       | 1       |
|                                   | 3.1- 4  | 2       | 2       | 3       | 1       | 1       |         |         |
|                                   | 2.1- 3  |         |         |         | 1       |         |         |         |
|                                   | 1.1- 2  |         | 1       |         |         |         |         |         |
|                                   | 0- 1    |         |         |         |         |         |         |         |
|                                   |         | 1.0-1.5 | 1.6-2.0 | 2.1-2.5 | 2.6-3.0 | 3.1-3.5 | 3.6-4.0 | 4.1-4.5 |
| GRADE                             |         |         |         |         |         |         |         |         |

The following method for determining the toughness of the strings was developed:

Remove a string from the pod and immediately fasten one end to a clamp weighing one-half pound. Holding the string so that there is about one-half inch between the fingers and the weight, lift gently. If any one-half inch portion of the string will support the weight for at least 5 seconds, regard it as a tough string.

While all of the samples were not examined for tough strings, an examination of the majority of them indicated that there should not be more than one tough string for each two ounces of drained weight of beans. For example, Inv. 13204-B-g, consisting of distinctly mature Kentucky Wonder beans, was found to contain 3 tough strings per two ounces of drained beans. The fairly tender Kentucky Wonder beans from the same lot contained 1 tough string per two ounces of drained beans, and the tender beans only 1 tough string to six ounces of drained beans. Numerous samples of tender and fairly tender beans were found to contain no tough strings when examined by this procedure.

#### SUMMARY

Methods have been developed to determine the maturity of canned snap (stringless) beans. Results obtained from 104 samples representing seven different varieties from as many different states indicate that standard beans should contain not more than 0.08 per cent fibrous material in the pods, 6 per cent seeds, and 7 per cent alcohol-insoluble solids; also that there should not be more than one tough string for each 2 ounces of drained weight of beans.

The writers express grateful appreciation to L. M. Beacham, Jr., and J. I. Palmore of the U. S. Food and Drug Administration for their assistance in the analytical work.

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### STUDIES RELATIVE TO THE ESTIMATION OF VITAMIN D

#### V. ROENTGEN (X-) RAY DIAGNOSIS AND ASH DETERMINATION OF BONE CALCIFICATION, AND BLOOD MINERAL ANALYSES IN WHITE LEGHORN CHICKS

By HENRY A. HALVORSON and LAWRENCE L. LACHAT (Feed Laboratory, Department of Agriculture, Dairy and Food, St. Paul, Minn.)

Expansion of the regulatory work in several state laboratories to include bioassays for vitamin D content of poultry supplements claimed to possess high antirachitic value soon necessitated investigations concerning the development of suitable testing methods. In 1934 (Griem) the Association tentatively adopted a preventive biological method (1), applicable to fish body and liver oils, and their extracts or derivatives, and to other materials used to enhance the vitamin D content of feeds, but not applicable to irradiated ergosterol and irradiated yeast, unless these products are recommended for poultry feeding. The authors (2), (3), (4), (5), (6) and others (Supplee (7), Griem (8), Griem *et al.* (9)) have recently reported several studies of fundamental factors affecting the accuracy of tests by this method and have presented data of value for improving and shortening the procedure.

The purpose of the present paper is to describe briefly (with presentation of part of the data) an extensive investigation conducted in this laboratory to determine the relative value of several different criteria for the interpretation of results, and to ascertain whether the length of the feeding period may be reduced. Further supplementary studies of a similar nature in this respect are planned in future experiments. Only such conclusions will be drawn as are apparent from cursory examination of the data, since subsequent papers deal with the experimental results in greater detail.

The work of Waddell and Rohdenburg (10), showing that irradiated cholesterol is responsible for greater antirachitic potency than is irradiated ergosterol for preventing rickets in chickens, and the report published by Bills and associates (11), stating that there is a difference in the effectiveness for the chicken of the natural vitamin D of some fish liver oils as measured by the U.S.P. (International) rat unit, constrained the writers to plan and carry out these experiments.

#### PRESENTATION OF DATA

In May and June 1935, lots of day-old, single comb white Leghorn chicks were secured commercially from the hatchery that previously had supplied birds for tests of vitamin D supplements reported by this laboratory (12). The chicks as received were divided into twelve groups of 100 each, each group further subdivided into two pens of 50 each, and corre-

TABLE 1.—Units eaten by each chick per 100 grams of feed consumed

| GROUP NO. | SUPPLEMENT                 | AMOUNT          | SOURCE  | U.S.P. VITAMIN D UNITS PER 100 GRAMS RATION |
|-----------|----------------------------|-----------------|---|---|
|           |                            | <i>per cent</i> |   |   |
| 1         | Corn oil                   | 1.00            | Household corn oil                                  | 0   |
| 2         | Poor grade cod-liver oil   | 0.50            | Composite sample of several commercial products     | ?   |
| 3         | Sardine (pilchard) oil     | 0.50            | " " " "   | ?   |
| 4         | Concentrated cod-liver oil | 0.13            | " " " "   | ?   |
| 5         | High-grade cod-liver oil   | 0.50            | " " " "   | ?   |
| 6         | Basal ration irradiated*   | 100.00          | Mixed with 1.00% corn oil and irradiated by authors | ?   |
| 7         | Irradiated ergosterol      | 0.28            | Dr. C. E. Bills, Mead Johnson & Co.                 | 703   |
| 8         | Irradiated ergosterol      | 0.56            | " " " "   | 1406  |
| 9         | Irradiated yeast           | 0.25            | Dr. C. A. Smith, Standard Brands, Inc.              | 630-765                                     |
| 10        | Irradiated yeast           | 0.50            | " " " "   | 1260-1530                                   |
| 11        | Standard cod-liver oil     | 0.28            | Dr. E. M. Nelson, U. S. Department of Agriculture   | 24  |
| 12        | Irradiated cholesterol     | 0.28            | Dr. J. Waddell, Acetol Products Co.                 | 28  |

\* Method of irradiation described in a subsequent paper, *This Journal*, 19, 637 (1936).

sponding pens fed identical diets and otherwise treated in similar manner, thus serving as like controls. The basal ration used was that specified in the A.O.A.C. method, augmented in the case of the negative controls (Group 1) and Group 6 with 1 per cent corn oil, and in the other ten groups with the supplement shown in Table 1. In Groups 2, 3, 4, 5, 7, 8, 11, 12, sufficient corn oil was used to make the total added oil 1 per cent.

Table 2 presents the average bone ash percentages of the dried, fat-free tibiae and magnesium, calcium, and inorganic phosphorus contents of plasma from pooled-blood samples of chicks selected from each group, when they were one day, one, two, three, and four weeks old. The reported data on bone ash are in nearly all cases the average results from ten tibiae or more, which were ashed and otherwise treated according to the technic described by Lachat (3). The calcium, phosphorus, and magnesium data are results of determinations made on plasma from a composite blood sample from 10-12 one-day-old chicks selected at five different times during the course of the experiment. When the birds were one week old, these determinations were made with three chicks near the average in weight for calcium and magnesium, and with three of average weight for inorganic phosphorus. The mineral analysis when the birds were two and three weeks old was made on pooled-blood samples from two chicks of average weight in the calcium-magnesium determinations, and

*Table to accompany Plate 34*

| BIRD NO. | X-RAY DIAGNOSIS<br>SCALE 1-10 | BONE ASH        | WEIGHT                        |
|----------|-------------------------------|-----------------|-------------------------------|
|          |                               | <i>per cent</i> | <i>grams (in parentheses)</i> |
| 5235     | 2                             | 29.88           | light (96)                    |
| 5248     | 3                             | 28.87           | average (114)                 |
| 5258     | 3                             | 31.45           | heavy (140)                   |
| 5305     | 3                             | 28.21           | average (111)                 |
| 5313     | 2                             | 30.94           | light (105)                   |
| 5316     | 1                             | 26.13           | heavy (148)                   |
| Average  | 2.3                           | 29.25           |                               |

*Table to accompany Plate 35*

| BIRD NO. | X-RAY DIAGNOSIS<br>SCALE 1-10 | BONE ASH        | WEIGHT                        |
|----------|-------------------------------|-----------------|-------------------------------|
|          |                               | <i>per cent</i> | <i>grams (in parentheses)</i> |
| 5333     | 9                             | 46.76           | light (175)                   |
| 5353     | 10                            | 46.10           | heavy (224)                   |
| 5364     | 10                            | 46.92           | average (187)                 |
| 5391     | 9                             | 46.79           | average (174)                 |
| 5400     | 10                            | 45.79           | heavy (226)                   |
| 5419     | 10                            | 46.80           | light (158)                   |
| Average  | 9.7                           | 46.53           |                               |



PLATE 34 --Roentgen ray diagnosis of calcification in chick tibiae (bird numbers reading from left to right) when birds four weeks old received 1.00% corn oil as a supplement to A.O.A.C. ration.

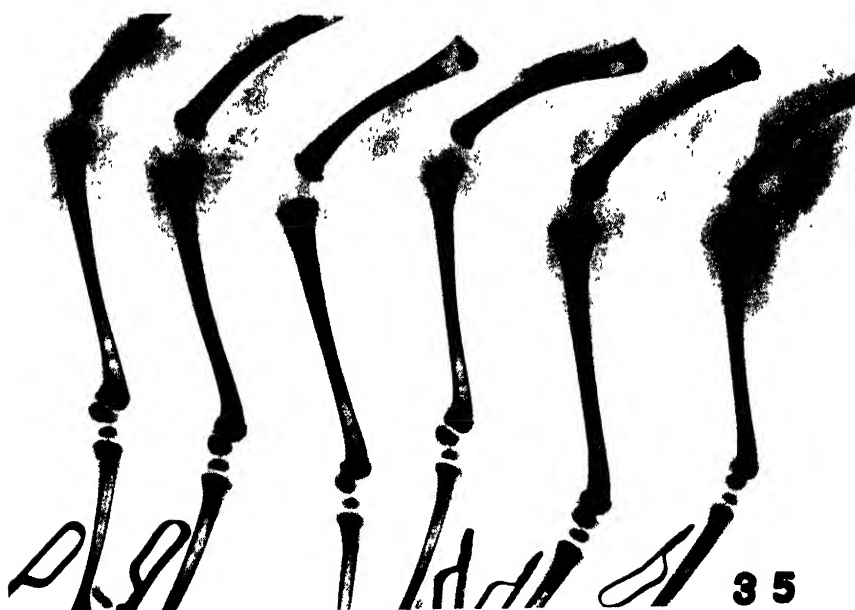


PLATE 35 --Roentgen ray diagnosis of calcification in chick tibiae (bird numbers reading from left to right) when birds four weeks old received 0.28% irradiated cholesterol as a supplement to A.O.A.C. ration



PLATE 36 --Roentgen ray diagnosis of calcification in chick tibiae (bird numbers reading from left to right) when birds four weeks old received 0.28% cod-liver oil as a supplement to A.O.A.C. ration



PLATE 37. Roentgen ray diagnosis of calcification in chick tibiae (bird numbers reading from left to right) when birds four weeks old received 0.28% irradiated ergosterol as a supplement to A.O.A.C. ration.

on two other chicks for estimation of phosphorus. When the birds were four weeks old, one chick of average weight furnished sufficient blood for the calcium-magnesium analysis, and one for the phosphorus determination. The technic used is given in detail elsewhere, Perish *et al.* (13).

Results are shown for the four different kinds of analysis. The analysts making the determinations were at no time aware of the identity of the specimens and had no means of recognizing paired samples. The agreement obtained in a majority of cases between identical determinations warrants the conclusion that where differences occur they may properly be assigned to (1) differences in the composition of the blood or bone of the individuals from which the samples were drawn, or (2) errors inherent in analytical methods used.

The data on mineral constituents of the blood, and the bone ash percentages given in Table 2 are presented graphically by Fig. 1. The maximum-minimum calcium and inorganic phosphorus contents of the blood plasma and the maximum-minimum bone ash percentages of the various groups at one day, one, two, three, and four weeks of age are plotted at the right-hand side of the figure.

To supplement the data presented in Table 2 and Fig. 1, and to observe the extent of correlation between bone ash results and Roentgen (X-)

Table to accompany Plate 56

| BIRD NO. | X-RAY DIAGNOSIS<br>SCALE 1-10 | BONE ASH        | WEIGHT                        |
|----------|-------------------------------|-----------------|-------------------------------|
|          |                               | <i>per cent</i> | <i>grams (in parentheses)</i> |
| 5428     | 7                             | 38.62           | heavy (203)                   |
| 5451     | 6                             | 40.99           | average (172)                 |
| 5457     | 7                             | 37.14           | light (129)                   |
| 5471     | 4                             | 34.46           | light (135)                   |
| 5485     | 8                             | 43.17           | heavy (203)                   |
| 5513     | 9                             | 44.59           | average (166)                 |
| Average  | 6.8                           | 39.83           |                               |

Table to accompany Plate 57

| BIRD NO. | X-RAY DIAGNOSIS<br>SCALE 1-10 | BONE ASH        | WEIGHT                        |
|----------|-------------------------------|-----------------|-------------------------------|
|          |                               | <i>per cent</i> | <i>grams (in parentheses)</i> |
| 5058     | 9                             | 42.34           | average (202)                 |
| 5067     | 10                            | 43.89           | heavy (216)                   |
| 5080     | 9                             | 44.52           | average (184)                 |
| 5088     | 9                             | 41.10           | heavy (230)                   |
| 5095     | 10*                           | 38.94*          | heavy (160)                   |
| 5103     | 10                            | 44.73           | light (177)                   |
| Average  | 9.4                           | 43.32           |                               |

\* Bird 3 weeks old not averaged with other specimens.



ray diagnosis of calcification, chicks of various sizes and ages were selected, the tibiae from these chicks (which generally included 1 light-, 2 average- and 1 heavy-weight birds or multiples thereof, from each group

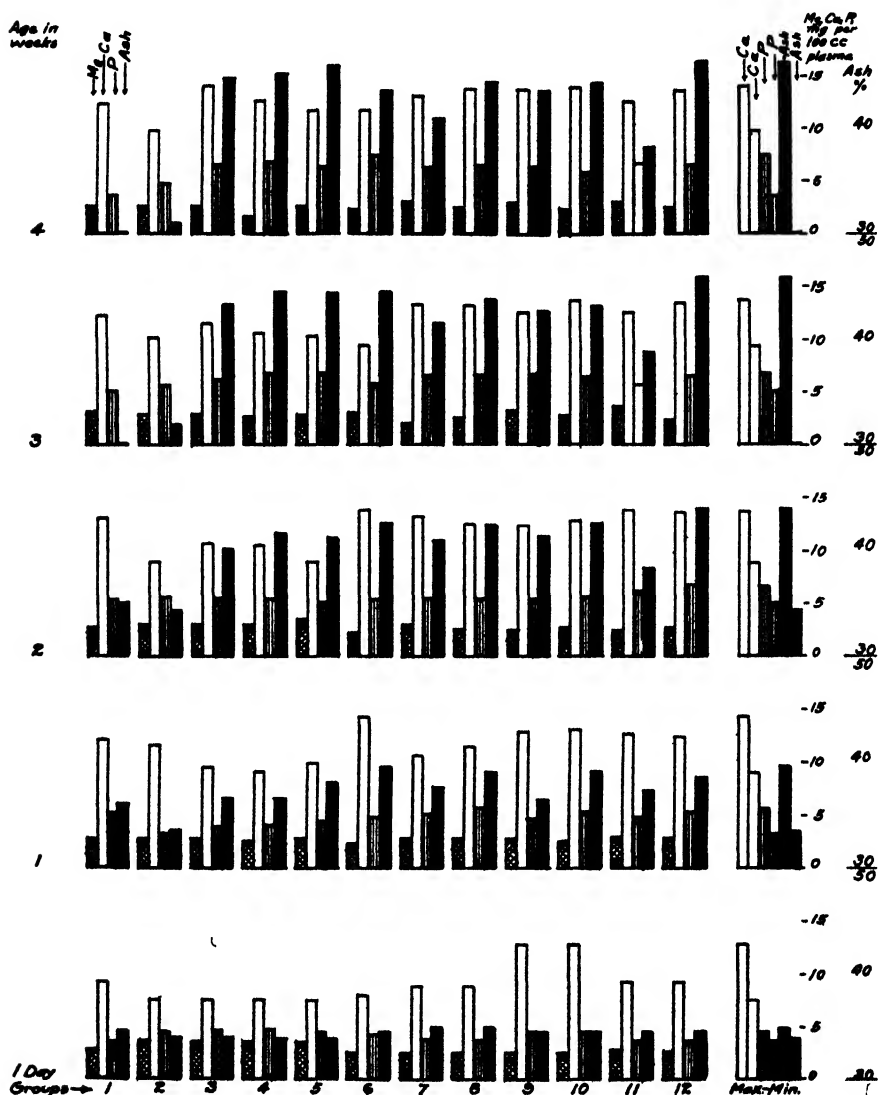


FIG. 1.—Diagram shows magnesium, calcium, and inorganic phosphorus in 100 cc. of blood plasma, and average ash contents of dried, fat-free tibiae of white Leghorns day old to four weeks of age fed various forms of vitamin D.

from one day to four weeks of age) being preserved in 50 per cent ethyl alcohol while awaiting examination. X-ray photographs (comprising pictures of 289 individual tibiae grouped on 52 plates) determining the den-

sity of calcium salts deposited in the bone, as observed from photographs and interpreted by a scale of 1-10, were made possible through the courtesy of A. C. Richardson of the California Packing Corporation. Space permits the reproduction of only four of the photographic plates illustrating the utility of this type diagnosis in comparison with the bone ash criterion. The data listed with Plates 34, 35, 36, and 37 include the bird number, X-ray diagnosis (Richardson), body weight, and bone ash percentage. Reference to Table 1 reveals the units eaten by each chick per 100 grams of feed consumed. To illustrate: The chicks whose tibiae were photographed on Plate 35 consumed only 28 units of vitamin D per 100 grams ration in the form of irradiated cholesterol, compared with 24 units from cod-liver oil consumed by birds whose tibiae are illustrated in Plate 36, and 703 units of vitamin D from irradiated ergosterol whose leg-bones were X-rayed and photographed in Plate 37.

#### DISCUSSION OF DATA

Although there is some variation in the magnesium content of the blood plasma of chicks, it is readily apparent from examination of Fig. 1 and Table 2 that this constituent is not influenced consistently either by age of the birds or amount and kind of vitamin D supplement used. Fig. 1 also shows that differences between the maximum and minimum plasma calcium are approximately the same regardless of age. It will be noted that Group 1 (fed the basal ration containing corn oil) shows nearly as much plasma calcium at the various ages as the maximum of the other groups. Apparently, then, the addition of vitamin D in the form of either fish liver or body oils and irradiated substances does not greatly increase the content of plasma calcium under conditions imposed by the A.O.A.C. method. The chicks in Group 2 (fed the basal ration supplemented by a composite sample of poor grade cod-liver oils) were the only ones that showed less than 10 mg. of calcium per 100 cc. plasma at four weeks of age. No great significance can be attached to this, however, since Group 11, which was fed a ration supplemented with cod-liver oil of low vitamin D potency, yielded a plasma calcium content nearly as great as the maximum at this age. It is also evident that plasma calcium in chicks of various ages is somewhat positively correlated with bone ash determinations of the tibia. It should be noted that whereas the bone ash data (as represented by black columns in Fig. 1) are averages of individual determinations on 20 or more tibiae excepting the day-old birds (averages on 10 or more), the plasma calcium contents are mostly averages of two determinations on pooled samples from only a few chicks.

The difference between the maximum and minimum inorganic phosphorus of blood plasma at the different ages is least in day-old and greatest in four-week-old birds, as will be noted from the graphs of Fig. 1. At one week of age, the lowest phosphorus content is associated with the lowest bone ash (see Group 2). At four weeks of age, a similar condition

prevails with respect to Groups 1 and 2. While the group showing the highest percentage of bone ash does not necessarily have the greatest amount of inorganic phosphorus, there seems to be a relation between high bone ash and an increased content of plasma phosphorus. Further reference to the data in Table 2 shows that the inorganic phosphorus approaches a constant in the second and third week of the chick's life, being then from 5 to 7 mg. per 100 cc. of plasma. From the second to fourth week, the groups receiving inadequate quantities of vitamin D (as evidenced by the low bone ash contents in Groups 1 and 2) exhibit decreasing amounts of inorganic phosphorus in the blood plasma.

In this connection Elvehjem and Kline(14) have reported the calcium and inorganic phosphorus content in whole blood of chicks under varying conditions to six weeks of age, rather than the amounts of these elements in plasma as recorded in this investigation. While they fed a constant calcium percentage of 0.91 in the ration and made a series of tests on a low phosphorus basis of 0.51 per cent and on a high basis of 0.70 per cent (thus varying the Ca:P ratio from 1.8:1 to 1.3:1), the writers maintained in their diet a uniform mineral content of 0.81–0.86 per cent of calcium and 0.68–0.71 per cent of phosphorus, with the Ca:P ratio averaging 1.2:1. The above differences in procedure will no doubt account partly for differences existing between the conclusions of Elvehjem and Kline and those of the writers, with respect to blood calcium. It was also found that absence of vitamin D from the ration had a small, but definite depressing effect on the calcium content of blood plasma at least to four weeks of age in agreement with the data of Elvehjem and Kline for whole blood. The results obtained by the writers apparently are in agreement with those found by these investigators to the effect that omission of vitamin D from the low phosphorus ration affected only slightly the blood phosphorus values.

The foregoing discussion, supported by data from Tables 1 and 2 and Fig. 1, demonstrates the superiority of the bone ash criterion over that of the magnesium, calcium, and phosphorus of blood plasma as a means to determine the antirachitic activity of poultry supplements. The great difference displayed in bone ash contents between groups of chicks receiving adequate and inadequate amounts of vitamin is clearly seen in Fig. 1, no such spread existing between the data assembled for the other factors. Determinations of the mineral constituents of blood may have the advantage of brevity, but dependability and certainty of correct interpretation of results under these conditions belong largely to the bone ash method, especially when birds two, three, or four weeks of age are considered.

The data also confirm the conclusion of Lachat (15) that the unsupplemented Hart-Kline-Keenan (16) vitamin D-deficient diet (slightly modified and adopted as the A.O.A.C. ration) produces a low bone ash in

TABLE 2.—Ca, Mg, and inorganic P in 100 cc. blood plasma and ash content of dried, fat-free tibiae of white Leghorns, day old to four weeks of age

| GROUP NO. | 1 DAY OLD |      |          |      |     | 1 WEEK OLD |      |          |      |     | 2 WEEKS OLD |      |          |      |     | 3 WEEKS OLD |      |          |      |     | 4 WEEKS OLD |      |          |      |     |          |      |          |      |
|-----------|-----------|------|----------|------|-----|------------|------|----------|------|-----|-------------|------|----------|------|-----|-------------|------|----------|------|-----|-------------|------|----------|------|-----|----------|------|----------|------|
|           | BONE ASH  |      |          | P    | mg. | BONE ASH   |      |          | P    | mg. | BONE ASH    |      |          | P    | mg. | BONE ASH    |      |          | P    | mg. | BONE ASH    |      |          | P    | mg. | per cent |      |          |      |
|           | Mg        | Ca   | mg.      |      |     | Mg         | Ca   | mg.      |      |     | Mg          | Ca   | mg.      |      |     | Mg          | Ca   | mg.      |      |     | Mg          | Ca   | mg.      |      |     |          | Mg   | Ca       | mg.  |
|           | mg.       |      | per cent |      |     | mg.        |      | per cent |      |     | mg.         |      | per cent |      |     | mg.         |      | per cent |      |     | mg.         |      | per cent |      |     | mg.      |      | per cent |      |
| 1         | 2.8       | 9.2  | 3.5      | 34.5 |     | 2.8        | 12.4 | 6.1      | 37.0 |     | 2.9         | 14.3 | 5.9      | 35.2 |     | 3.6         | 12.3 | 5.5      | 30.0 |     | 2.7         | 11.5 | 3.7      | 30.1 |     | 2.7      | 11.5 | 3.7      | 30.1 |
|           | 2.8       | 9.2  | 3.5      | 34.5 |     | 2.8        | 12.0 | 4.3      | 35.1 |     | 2.7         | 11.8 | 5.0      | 34.7 |     | 2.5         | 12.0 | 4.7      | 30.2 |     | 2.5         | 12.8 | 3.3      | 30.3 |     | 2.5      | 12.8 | 3.3      | 30.3 |
| 2         | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.9        | 11.6 | 3.9      | 34.4 |     | 3.0         | 9.4  | 5.4      | 33.5 |     | 3.0         | 10.4 | 6.2      | 31.8 |     | 2.3         | 9.8  | 4.9      | 30.9 |     | 2.3      | 9.8  | 4.9      | 30.9 |
|           | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.9        | 11.3 | 2.5      | 32.7 |     | 3.0         | 8.4  | 5.6      | 35.0 |     | 2.5         | 9.7  | 5.0      | 31.9 |     | 3.0         | 9.9  | 4.6      | 30.9 |     | 3.0      | 9.9  | 4.6      | 30.9 |
| 3         | 3.5       | 7.6  | 4.5      | 33.9 |     | 3.0        | 9.7  | 2.9      | 36.2 |     | 3.0         | 9.2  | 5.5      | 38.8 |     | 2.7         | 12.6 | 6.1      | 43.4 |     | 2.3         | 14.7 | 6.4      | 44.8 |     | 2.3      | 14.7 | 6.4      | 44.8 |
|           | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.7        | 9.3  | 4.7      | 36.8 |     | 2.9         | 12.0 | 5.8      | 41.4 |     | 2.7         | 10.2 | 6.2      | 43.0 |     | 2.5         | 13.4 | 6.5      | 44.8 |     | 2.5      | 13.4 | 6.5      | 44.8 |
| 4         | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.4        | 8.7  | 3.8      | 36.4 |     | 3.1         | 11.1 | 5.2      | 41.6 |     | 2.6         | 10.2 | 7.6      | 44.5 |     | 1.9         | 13.5 | 7.1      | 45.4 |     | 1.9      | 13.5 | 7.1      | 45.4 |
|           | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.7        | 9.4  | 4.4      | 36.9 |     | 2.9         | 9.7  | 5.7      | 41.3 |     | 2.6         | 10.9 | 6.0      | 44.2 |     | 1.4         | 12.1 | 6.6      | 44.9 |     | 1.4      | 12.1 | 6.6      | 44.9 |
| 5         | 3.5       | 7.6  | 4.5      | 33.9 |     | 2.6        | 10.7 | 4.8      | 38.6 |     | 3.6         | 8.8  | 5.1      | 40.4 |     | 2.9         | 9.9  | 7.0      | 43.8 |     | 2.6         | 11.7 | 5.8      | 45.9 |     | 2.6      | 11.7 | 5.8      | 45.9 |
|           | 3.5       | 7.6  | 4.5      | 33.9 |     | 3.1        | 9.2  | 3.9      | 37.6 |     | 3.3         | 9.2  | 5.1      | 41.8 |     | 2.5         | 10.6 | 6.5      | 44.7 |     | 2.6         | 11.9 | 6.7      | 46.3 |     | 2.6      | 11.9 | 6.7      | 46.3 |
| 6         | 2.6       | 8.0  | 4.3      | 34.4 |     | 1.8        | 14.3 | 4.9      | 39.9 |     | 2.4         | 13.8 | 4.1      | 42.5 |     | 3.0         | 9.3  | 4.8      | 44.3 |     | 2.2         | 10.5 | 8.1      | 44.0 |     | 2.2      | 10.5 | 8.1      | 44.0 |
|           | 2.6       | 8.0  | 4.3      | 34.4 |     | 2.6        | 14.1 | 4.5      | 39.3 |     | 1.9         | 13.5 | 6.3      | 42.6 |     | 3.0         | 9.5  | 6.5      | 44.4 |     | 2.3         | 13.0 | 7.1      | 43.6 |     | 2.3      | 13.0 | 7.1      | 43.6 |
| 7         | 2.6       | 9.0  | 3.8      | 34.9 |     | 2.8        | 10.9 | 5.2      | 38.7 |     | 3.4         | 13.2 | 5.3      | 40.7 |     | 1.3         | 13.3 | 6.5      | 42.4 |     | 2.9         | 13.1 | 6.3      | 40.7 |     | 2.9      | 13.1 | 6.3      | 40.7 |
|           | 2.6       | 9.0  | 3.8      | 34.9 |     | 3.0        | 10.2 | 5.0      | 36.5 |     | 2.5         | 13.0 | 5.7      | 41.0 |     | 2.7         | 12.9 | 6.8      | 40.8 |     | 3.1         | 13.4 | 6.3      | 41.4 |     | 3.1      | 13.4 | 6.3      | 41.4 |
| 8         | 2.6       | 9.0  | 3.8      | 34.9 |     | 2.8        | 11.1 | 5.5      | 39.6 |     | 2.1         | 13.1 | 5.7      | 42.0 |     | 2.8         | 13.2 | 6.5      | 44.3 |     | 2.5         | 13.9 | 6.3      | 44.3 |     | 2.5      | 13.9 | 6.3      | 44.3 |
|           | 2.6       | 9.0  | 3.8      | 34.9 |     | 2.6        | 11.4 | 6.0      | 38.6 |     | 2.9         | 12.0 | 5.0      | 42.5 |     | 2.1         | 13.2 | 6.4      | 43.4 |     | 2.6         | 13.9 | 6.8      | 44.2 |     | 2.6      | 13.9 | 6.8      | 44.2 |
| 9         | 2.5       | 12.8 | 4.5      | 34.4 |     | 3.0        | 13.1 | 4.7      | 37.3 |     | 3.0         | 13.0 | 6.0      | 40.7 |     | 3.1         | 13.5 | 6.8      | 41.9 |     | 3.2         | 13.2 | 6.3      | 44.1 |     | 3.2      | 13.2 | 6.3      | 44.1 |
|           | 2.5       | 12.8 | 4.5      | 34.4 |     | 2.6        | 12.4 | 4.4      | 35.8 |     | 1.9         | 11.4 | 4.8      | 41.8 |     | 3.1         | 11.4 | 6.7      | 43.5 |     | 2.6         | 14.5 | 6.7      | 43.4 |     | 2.6      | 14.5 | 6.7      | 43.4 |
| 10        | 2.5       | 12.8 | 4.5      | 34.4 |     | —          | 12.3 | 5.6      | 38.9 |     | 2.8         | 13.1 | 5.8      | 42.8 |     | 2.9         | 13.6 | 6.1      | 43.4 |     | 1.9         | 13.7 | 6.2      | 44.5 |     | 1.9      | 13.7 | 6.2      | 44.5 |
|           | 2.5       | 12.8 | 4.5      | 34.4 |     | 2.6        | 13.8 | 4.9      | 39.4 |     | 2.8         | 12.5 | 5.4      | 42.2 |     | 2.7         | 14.1 | 6.8      | 43.1 |     | 2.7         | 14.5 | 5.7      | 44.0 |     | 2.7      | 14.5 | 5.7      | 44.0 |
| 11        | 2.8       | 9.2  | 3.5      | 34.5 |     | 3.2        | 12.5 | 5.2      | 37.4 |     | 3.9         | 13.3 | 6.2      | 39.5 |     | 3.8         | 13.3 | 4.9      | 38.4 |     | 2.5         | 12.7 | 7.0      | 38.1 |     | 2.5      | 12.7 | 7.0      | 38.1 |
|           | 2.8       | 9.2  | 3.5      | 34.5 |     | 2.6        | 12.6 | 4.6      | 37.4 |     | 1.0         | 14.1 | 6.2      | 37.3 |     | 3.2         | 11.6 | 6.5      | 39.4 |     | 3.6         | 12.5 | 6.5      | 38.3 |     | 3.6      | 12.5 | 6.5      | 38.3 |
| 12        | 2.8       | 9.2  | 3.5      | 34.5 |     | 3.0        | 12.7 | 4.9      | 39.6 |     | 1.9         | 13.0 | 6.4      | 43.9 |     | 2.7         | 13.3 | 6.3      | 45.9 |     | 1.9         | 13.5 | 6.7      | 46.3 |     | 1.9      | 13.5 | 6.7      | 46.3 |
|           | 2.8       | 9.2  | 3.5      | 34.5 |     | 2.4        | 12.0 | 5.5      | 37.8 |     | 3.3         | 14.2 | 7.0      | 44.0 |     | 1.8         | 13.5 | 6.8      | 45.7 |     | 3.0         | 13.8 | 6.4      | 46.4 |     | 3.0      | 13.8 | 6.4      | 46.4 |

young chicks in a period of two weeks. Fig. 1 shows that there are sufficient differences between the bone ash percentages in groups receiving adequate and inadequate amounts of vitamin D at three, and possibly even at two weeks of age, to properly sustain conclusions regarding the antirachitic potency of poultry supplements.

#### X-RAY DIAGNOSIS

Determination of the efficacy of vitamin D supplements by X-ray diagnosis of the leg bones has the advantage of being time- and labor-saving and does not entail the destruction of the chick. The disadvantage of this procedure, apart from the often prohibitive cost of equipment and the fact that few feed control laboratories have a trained personnel for making such tests, is its inferiority compared with the bone ash method for accurate interpretation of results. Roentgen ray diagnosis is influenced by the element of human error, since comparisons must be made with a standard that has been obtained previously as a result of bone ash determinations, the judgment of two different analysts on a tibia sample very easily varying two points or more, resulting in an error of the standard of at least 20 per cent. In the bone ash method, however (assuming that concordant results may be obtained in various laboratories, a fact already amply demonstrated (3)), the analyst has the advantage of a definite figure for each tibia, and this is not subject to the same magnitude of error as that mentioned above and obtained when Roentgen rays are used. The extent of the relation between the X-ray diagnosis of calcification and bone ash results is satisfactorily illustrated by the data accompanying the four Roentgen ray photographs. A comparison of results on the tibiae reproduced in two plates will show that X-ray diagnosis indicates very nearly as satisfactory calcification of the bones in Plate 37 as that of those in Plate 35. In contrast, when the criteria recommended by the writers for interpreting results<sup>1</sup> are used the bone analysis shows normal calcification in all tibiae on Plate 35, but the same growth and calcification data (see Group 7 in Table 2 and Fig. 1) show that both as individuals and as a group the tibiae in Plate 37, as well as those of other individuals in this group, were decidedly sub-normal.

#### SUMMARY AND CONCLUSIONS

The investigation described involved feeding experiments on twelve groups of single-comb white Leghorn chicks (each group consisting of two controls of 50 birds each) from day old to four weeks of age, the groups being fed the basal A.O.A.C. ration containing various kinds and amounts of vitamin D supplements. Roentgen ray diagnosis and bone ash determination of calcification in the tibiae and analyses of blood plasma for magnesium, calcium, and inorganic phosphorus at day-old and four

<sup>1</sup> A vitamin D carrier shall be adjudged as showing satisfactory calcification according to the A.O.A.C. method of assay only when average bone ash content exceeds 45% and growth rate is normal, and when 80% of the total number of birds in the assay group are in excess of 45% bone ash (5).

weekly intervals under the experimental conditions appear to support the following conclusions:

(1) The magnesium or calcium content of blood plasma is not influenced consistently and inorganic phosphorus only slightly either by age of the chick or amount and kind of vitamin-D supplement used.

(2) Bone ash determination of the tibia is superior to Roentgen ray diagnosis of bone structure and to magnesium, calcium, and inorganic phosphorus of blood plasma of chicks as a criterion for estimating vitamin-D content of poultry supplements.

(3) The data confirm the results obtained by Lachat (15) that the unsupplemented A.O.A.C. vitamin-D deficient diet produces a low bone ash in young chicks as early as two weeks.

(4) The length of feeding period of the A.O.A.C. method may be shortened from 4 to 3, and possibly 2 weeks.

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- (16) *Science*, 73, 710-1 (1931).

## STUDIES RELATIVE TO THE ESTIMATION OF VITAMIN D

### VI. COMPARATIVE VITAMIN-D REQUIREMENT OF THE CHICK FOR SARDINE (PILCHARD), CONCENTRATED, AND COD-LIVER OILS, IRRADIATED YEAST, IRRADIATED ERGOSTEROL, AND IRRADIATED CHOLESTEROL

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The amount of various substances containing vitamin D required for satisfactory growth and adequate calcification of the bones in the chick has been reported in comparative experiments by Murphy, Hunter, and Knandel (1) for cod-liver oil fortified in vitamin D, by Carver and co-workers (2), Couch *et al.* (3), and Lachat and Halvorson (4)-(5) for un-

diluted cod-liver oil; and by Bethke, Record and Kennard (6) for irradiated yeast, irradiated ergosterol, and cod-liver oil, the latter substance being both undiluted and fortified with vitamin D. Recently, Waddell and Rohdenburg (7), using cod-liver oil as a standard of reference, observed that irradiated cholesterol, alone of activated compounds, possesses the same vitamin activity (unit-equivalence) for both chicks and rats. In part, these studies suggest that this requirement is largely dependent upon the supply and ratio of calcium, phosphorus, and vitamin D in the ration employed and that many irradiated substances do not exhibit the same measure of rickets prevention for the two species.

These and other considerations prompted the writers to plan several studies by the A.O.A.C. method of bioassay, Griem (8), because at present very little information is available correlating irradiated substances and their derivatives with calcification in both young rats and chicks. A report of the findings of the writers relating particularly to a number of vitamin D substances bioassayed in this manner, employing several different criteria in interpreting results, has been published separately (9).

In the present study the effectiveness of various kinds and amounts of antirachitic substances and their possible evaluation in preventing avian rickets<sup>1</sup> has been determined when growth and feed consumption records supplement the interpretative criteria of bone ash determination, mineral analysis of blood plasma, and diagnosis of bone calcification by means of Roentgen rays.

#### PRESENTATION OF DATA

Day-old, single-comb white Leghorns, obtained commercially and housed in electrically-heated battery brooders equipped with raised wire grids, were given *ad lib.* distilled water and the A.O.A.C. vitamin-D deficient ration, refer to Hart and associates (10), Lachat *et al.* (11). Irradiated yeast or one per cent of corn oil (Mazola) containing the various oil additions supplemented this basal diet. Preventive technic according to the procedure of A.O.A.C. bioassay was used throughout. All chicks were started within a total time of six weeks in order that the variation due to possible seasonal or other effects should be quite small.

Birds near the average in size were used for these experiments and were selected at weekly intervals from a larger number available at the start of each feeding trial, for determining tibia ash content and mineral analysis of the blood plasma, the selection being chiefly on the basis of weight; as for example, 3 light, 6 average, and 3 heavy, the remainder being continued on feed until the next weekly weighing period, when the procedure was repeated.

When day-old, one, two, three or four weeks of age, surviving birds were destroyed by breaking the neck, pooled samples of blood were taken

<sup>1</sup> Mammalian rickets as it occurs in rats and infants is a disease probably not analogous to a similar rickets-like syndrome produced in young chicks in these experiments. It seems probable that a combination of both osteoporosis and rickets exists when chicks subsist on the A.O.A.C. diet without added vitamin D.

for mineral analysis, the left tibia was ashed by the standard procedure, Lachat (12), and the right leg of each representative bird was placed in 50 per cent  $C_2H_5OH$  for Roentgen ray diagnosis of the extent and density of their calcification. Exposure of the latter specimens to Roentgen rays was subsequently accomplished by A. C. Richardson, California Packing Corporation, San Francisco (for a partial presentation of these data see Halvorson and Lachat (9)). Samples of chicken blood plasma were analyzed for calcium, inorganic phosphorus, and magnesium by the technic recently described by Perish and associates (13). Unfortunately, with the younger birds it was often necessary to obtain blood samples from more than one host, and this caused pooling of blood specimens obtained from representative birds near the average in weight.

TABLE 1.—*Vitamin potency in U.S.P. units of various substances fed chicks by A.O.A.C. method*

| LOT NO. | AMOUNT OF SUPPLEMENT ADDED TO BASAL RATION | U.S.P. VITAMIN D<br>UNITS PER 100 g.<br>RATION | Ca              | P               |
|---------|--|--|-----------------|-----------------|
|         | <i>per cent</i>                            |  | <i>per cent</i> | <i>per cent</i> |
| 1       | 1.00 corn oil                              | 0.0  |                 |                 |
| 2       | 1.00 corn oil                              | 0.0  | 0.83            | 0.69            |
| 3       | 0.50 low-vitamin D cod-liver oil§          | ?  |                 |                 |
| 4       | 0.50 low-vitamin D cod-liver oil§          | ?  | 0.83            | 0.68            |
| 5       | 0.50 sardine (pilchard) oil†               | ?  |                 |                 |
| 6       | 0.50 sardine (pilchard) oil†               | ?  | 0.85            | 0.68            |
| 7       | 0.13 concentrated cod-liver oil‡           | ?  |                 |                 |
| 8       | 0.13 concentrated cod-liver oil‡           | ?  | 0.84            | 0.68            |
| 9       | 0.50 high-vitamin D cod-liver oil**        | ?  |                 |                 |
| 10      | 0.50 high-vitamin D cod-liver oil**        | ?  | 0.86            | 0.68            |
| 11      | Same as lots 1 and 2 ration, irradiated    | ?  |                 |                 |
| 12      | Same as lots 1 and 2 ration, irradiated    | ?  | 0.84            | 0.68            |
| 13      | 0.28 irradiated ergosterol*                | 703  |                 |                 |
| 14      | 0.28 irradiated ergosterol*                | 703  | 0.81            | 0.70            |
| 15      | 0.56 irradiated ergosterol*                | 1406   |                 |                 |
| 16      | 0.56 irradiated ergosterol*                | 1406   | 0.83            | 0.70            |
| 17      | 0.25 irradiated yeast*                     | 630-765  |                 |                 |
| 18      | 0.25 irradiated yeast*                     | 630-765  | 0.82            | 0.69            |
| 19      | 0.50 irradiated yeast*                     | 1260-1530                                      |                 |                 |
| 20      | 0.50 irradiated yeast*                     | 1260-1530                                      | 0.83            | 0.71            |
| 21      | 0.28 cod-liver oil*                        | 24   |                 |                 |
| 22      | 0.28 cod-liver oil*                        | 24   | 0.84            | 0.70            |
| 23      | 0.28 irradiated cholesterol*               | 28   |                 |                 |
| 24      | 0.28 irradiated cholesterol*               | 28   | 0.84            | 0.71            |

\* Bioassayed by U. S. Pharmacopoeia method with rats as follows: Irradiated ergosterol, irradiated yeast, irradiated cholesterol and cod-liver oil by C. E. Bills, C. A. Smith, J. Waddell, and E. M. Nelson, and donated for these experiments by Mead Johnson & Company, Standard Brands Inc., Acetol Products Inc., and the U. S. Department of Agriculture, respectively.

† Prepared from 4 oils of fair vitamin potency bioassayed by A.O.A.C. method.

‡ Prepared from 3 concentrates, bioassayed individually by A.O.A.C. method and shown to be adequate for satisfactory calcification when eaten by chicks at the 0.13 % level.

§ Prepared from 5 commercial oils of low vitamin content previously bioassayed in this laboratory by A.O.A.C. method.

\*\* Prepared from 4 oils of high vitamin content bioassayed by A.O.A.C. method.



Experimental chicks were equally divided into groups. The various additions to the ration, mineral analyses, vitamin potency of the supplements fed, and group numbers are recorded in Table 1. Irradiating the basal ration was accomplished by exposing the well-mixed ingredients in shallow pans, one-quarter inch deep, with stirring at 5-minute intervals at a distance approximately 4 feet from the carbon arc. The unit was operated at 220 volts to produce 60 amperes at 50 volts across a U carbon (National Carbon Company Model 49 M) for 20 minutes.<sup>1</sup> Calcium and phosphorus content of the rations employed (Table 1) were determined by the method of Morris, Nelson and Palmer (14), and from these data it is apparent that very little variation existed. Table 2 presents the average bone ash of dried fat-free tibiae, initial and final weights, feed consumption records, and the contents of plasma minerals as they occur in four-week-old birds having access to several different antirachitic substances.

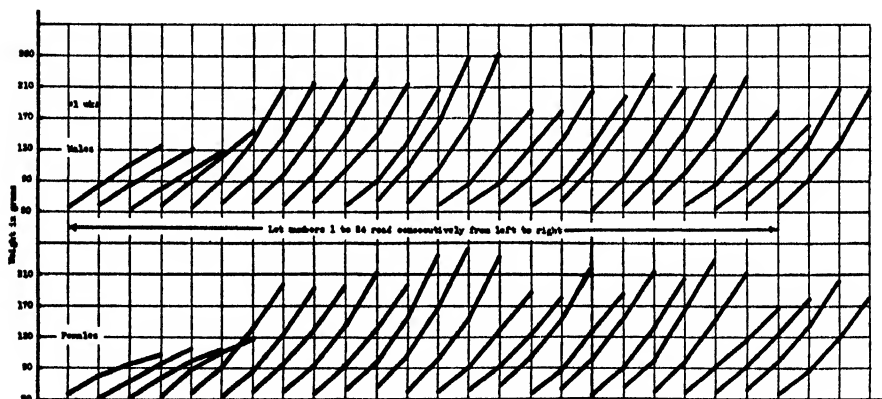


FIG. 1.—Separate growth records shown by sexes during first to fourth week of experiment.

It will be observed that increase in weight was almost perfectly correlated positively with the amount of feed consumed ( $r$  upon calculation = 0.991 with standard error of 0.004, Fisher (15)), the correlation being associated with average ash content varying from 30 to 46 per cent. The calcium, magnesium, and phosphorus contents of the plasma were but poorly correlated with ash content, and apparently show no definite detectable trend. This lack of agreement is somewhat surprising in view of the belief that the mineral moiety of blood plasma usually follows calcification in the growing organism. Thus, when the ash of bones is deficient, blood minerals are likewise expected to be low, and when calcification is normal the minerals of the blood bathing the bones may also be

<sup>1</sup> Thanks are due the American Crystal Sugar Company for placing their equipment at the disposal of the writers for the weekly irradiation of feed.

presumed to be normal in amount. The plasma phosphorus evidently follows bone calcification more closely than does plasma calcium, although discrepancies between these phosphorus results and ash content also seem apparent from the data.

Growth records are reported separately by sexes in Fig. 1, the total individuals of all groups being 132 males and 147 females. Fig. 2 exhibits

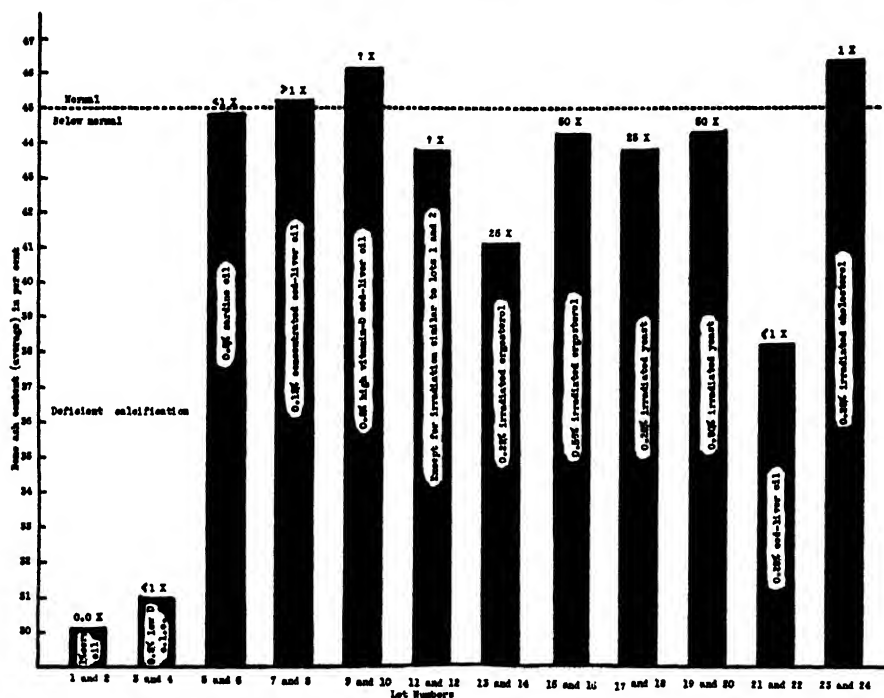


FIG. 2.—Comparative calcification produced in the various lots when the average of Lots 23 and 24, those which received 28 U.S.P. vitamin D units from irradiated cholesterol per 100 grams A.O.A.C. ration, is used as a standard of reference (1 X = 28 units).

graphically the comparative calcification produced by the various substances eaten, a bone ash content of 45 per cent being used for normally calcified birds. It will be observed that only groups 7-8, 9-10, and 23-24, those given concentrated cod-liver oil, high vitamin-D cod-liver oil, and irradiated cholesterol, respectively, had bones normally calcified. All other groups were below normal in this respect, with average ash content ranging from approximately 30 to just below 45 per cent.

#### DISCUSSION

Comparative requirement of the chick for vitamin D, in addition to that naturally present in the A.O.A.C. ration supplemented by a substance (corn oil) practically devoid of calcifying ability, is shown for

TABLE 2.—*Mineral analyses of blood and calcification, produced by various substances given chicks*

| LOT NO. | SUPPLEMENT TO BASAL RATION   | NO. BIRDS SURVIVING | AVERAGE          |                | AVERAGE      |      | AVERAGE  |         | PER 100 CC. BLOOD PLASMA |            |  |
|---------|------------------------------|---------------------|------------------|----------------|--------------|------|----------|---------|--------------------------|------------|--|
|         |                              |                     | FEED CONSUMPTION | INITIAL WEIGHT | FINAL WEIGHT | AGE  | PER CENT | CALCIUM | MAGNESIUM                | PHOSPHORUS |  |
|         |                              |                     | GRAMS            | GRAMS          | GRAMS        |      |          | MG.     | MG.                      | MG.        |  |
| 1       | Corn oil                     | 13                  | 216              | 39             | 124          | 30.1 |          | 11.5    | 2.7                      | 3.7        |  |
| 2       | Corn oil                     | 14                  | 220              | 40             | 121          | 30.3 |          | 12.8    | 2.5                      | 3.3        |  |
| 3       | Low-vitamin D cod-liver oil  | 11                  | 228              | 39             | 120          | 30.9 |          | 9.8     | 2.3                      | 4.9        |  |
| 4       | Low-vitamin D cod-liver oil  | 12                  | 255              | 40             | 139          | 30.9 |          | 9.9     | 3.0                      | 4.6        |  |
| 5       | Sardine oil                  | 11                  | 339              | 40             | 204          | 44.8 |          | 14.7    | 2.3                      | 6.4        |  |
| 6       | Sardine oil                  | 11                  | 344              | 40             | 207          | 44.8 |          | 13.4    | 2.5                      | 6.5        |  |
| 7       | "Concentrated" cod-liver oil | 11                  | 353              | 39             | 207          | 45.4 |          | 13.5    | 1.9                      | 7.1        |  |
| 8       | "Concentrated" cod-liver oil | 11                  | 349              | 39             | 219          | 44.9 |          | 12.1    | 1.4                      | 6.6        |  |
| 9       | High-vitamin D cod-liver oil | 10                  | 334              | 39             | 203          | 45.9 |          | 11.7    | 2.6                      | 5.8        |  |
| 10      | High-vitamin D cod-liver oil | 12                  | 361              | 38             | 224          | 46.3 |          | 11.9    | 2.6                      | 6.7        |  |
| 11      | Ration irradiated            | 12                  | 391              | 37             | 243          | 44.0 |          | 10.5    | 2.2                      | 8.1        |  |
| 12      | Ration irradiated            | 11                  | 390              | 37             | 242          | 43.6 |          | 13.0    | 2.3                      | 7.1        |  |
| 13      | Irradiated ergosterol        | 13                  | 307              | 42             | 189          | 40.7 |          | 13.1    | 2.9                      | 6.3        |  |
| 14      | Irradiated ergosterol        | 12                  | 301              | 41             | 183          | 41.4 |          | 13.4    | 3.1                      | 6.3        |  |
| 15      | Irradiated ergosterol        | 12                  | 326              | 42             | 204          | 44.3 |          | 13.9    | 2.5                      | 6.3        |  |
| 16      | Irradiated ergosterol        | 11                  | 315              | 41             | 190          | 44.2 |          | 13.9    | 2.6                      | 6.8        |  |
| 17      | Irradiated yeast             | 11                  | 367              | 37             | 220          | 44.1 |          | 13.2    | 3.2                      | 6.3        |  |
| 18      | Irradiated yeast             | 11                  | 340              | 37             | 206          | 43.4 |          | 14.5    | 2.6                      | 6.7        |  |
| 19      | Irradiated yeast             | 11                  | 381              | 37             | 228          | 44.5 |          | 13.7    | 1.9                      | 6.2        |  |
| 20      | Irradiated yeast             | 11                  | 371              | 37             | 218          | 44.0 |          | 14.5    | 2.7                      | 5.7        |  |
| 21      | Cod-liver oil                | 12                  | 286              | 39             | 170          | 38.1 |          | 12.7    | 2.5                      | 7.0        |  |
| 22      | Cod-liver oil                | 12                  | 287              | 39             | 172          | 38.3 |          | 12.5    | 3.6                      | 6.5        |  |
| 23      | Irradiated cholesterol       | 12                  | 331              | 38             | 204          | 46.3 |          | 13.5    | 1.9                      | 6.7        |  |
| 24      | Irradiated cholesterol       | 12                  | 315              | 40             | 195          | 46.4 |          | 13.8    | 3.0                      | 6.4        |  |

various substances by Fig. 2. When reference is made to ash content and the decreased amounts of plasma calcium and phosphorus in Table 2, it is apparent that Groups 1-2 (given the basal diet plus corn oil) had a very limited supply of antirachitic vitamin. Provided the amount present is assumed to be a minimum, these two lots may be used as a basis of reference for graded additions of antirachitic substances supplementing the basal diet. When the calcifying potency is expressed on the basis of units per 100 grams ration, there are no figures available on which to determine correctly the potency of Lots 3-12, inclusive.

Of all the groups concerned, it is evident that the extra requirement for vitamin is least in Lots 23-24, which received irradiated cholesterol, and based on the activity secured by rat bioassays is 28 units per 100 grams of ration. The requirement for satisfactory growth and calcification compares favorably with previously reported figures (5) for the U. S. Pharmacopocia "Reference cod-liver oil." The demands for this factor when supplied by the latter product were satisfied by approximately 26.7 units per 100 grams of diet per chick for the season of the year in which these experiments may be compared.

From the data contained in the second and third columns of Table 1 and ash content of Table 2, it is apparent that Groups 3-4 and 5-6 (those receiving rations supplemented with vitamin D in the form of cod-liver and sardine oils having poor and fair calcifying ability, respectively) received less vitamin per unit of ration than did Groups 23-24 (fed irradiated cholesterol), although the rates of growth in the sardine and irradiated cholesterol lots were quite similar (Fig. 1). In several experiments the writers have found calcification to be characterized as subnormal when the bone ash for A.O.A.C. bioassay is below 45 per cent (Lachat and Halvorson (16)), as shown graphically by Fig. 2. Irradiating the ration in Lots 11-12 apparently induced a definite calcifying effect in certain ingredients constituting the negative control diet of Lots 1-2, but the calcification produced was slightly below normal. The content of plasma calcium and phosphorus seemed normal or nearly so, the values for the latter being among the highest reported for all groups. Also, in one of the lots receiving irradiated feed (No. 11 but not 12), the plasma Ca/P ratio was very similar to that for the ration eaten.

Irradiated ergosterol (Lots 15-16), even when fed to chicks in amounts approximately 50 $\times$  the vitamin D units present in the irradiated cholesterol group, failed to produce a normally calcified bone, although growth in these instances was nearly normal.<sup>1</sup> When 25 $\times$  the amount of vitamin present in the form of irradiated cholesterol was eaten by the birds in Lots 13-14, the calcification and increase in weight were both definitely below normal. Certain deductions of significance do not appear

<sup>1</sup> In these experiments the normal for weight increase has been arbitrarily placed at an average value of 150 g. per bird during 4-weeks' feeding of the diet, adequately supplemented by vitamin D.

possible from consideration of the blood mineral analysis. The feeding of irradiated yeast to Groups 17-20, inclusive, while causing fair growth in both males and females (Fig. 1) resulted in subnormal calcification. The blood plasma exhibited no noticeable decrease in amount of minerals, which may possibly be explained by the lack of sensitivity inherent in this criterion or by individual variability in the blood sample of birds from which the mineral analysis was derived. It appears that more than  $50\times$  the normal requirement of vitamin from irradiated yeast is essential for adequate calcification, at least when it is compared with the vitamin in the form of irradiated cholesterol.

It is further manifest that irradiated yeast is not superior in calcifying abilities to the same property of irradiated ergosterol when  $25\times$  or  $50\times$  the vitamin activity of irradiated cholesterol is eaten by these lots. The slight superiority of irradiated yeast at the  $25\times$  level as shown comparatively between Groups 17-18 and 13-14 lacks significance when groups receiving irradiated yeast and irradiated ergosterol at the  $50\times$  level are compared, as for example, between Groups 19-20 and 15-16.

Peculiarly, the growth of these experimental yeast-fed birds (in spite of the apparent lack of vitamin D) was quite satisfactory when compared with normal conditions in the other lots. There is some evidence, therefore, that feeding irradiated yeast apart from its possible deficiency of vitamin D stimulated appetite and this caused an increase in feed consumption. The significance of this observation is somewhat obscured by the fact that the ration itself contained a small amount of pure non-irradiated whole yeast. The possible appetite-stimulating effect resident in irradiated yeast would properly be measured by a restriction of feed to similar pairs of chicks in individual compartments that are otherwise treated exactly alike. It seems probable, however, that this response in appetite, causing increased weight gains in young chicks, may be more than offset by the chick's prompt response to an adequate supply of vitamin D with a corresponding increase in bone calcification.

In Lots 21-22, those given a cod-liver oil whose content of vitamin D had been accurately determined by the U. S. Pharmacopoeia method, the vitamin intake was somewhat deficient for favorable growth and adequate calcification. The data are of special significance in demonstrating that a very small amount of vitamin lacking from the ordinary diet is promptly apparent by the methods employed, and further, such deficiency may be evidenced by a decreased growth rate and faulty calcification. Of interest in this connection is a comparison of the requirement of these birds with those that subsisted on the U.S.P. reference cod-liver oil at a comparable period of time. The requirement for the latter was found to be about  $26+$  units per 100 grams of feed per chick.

In the foregoing discussion the relation between the amount of vitamin required from different substances has been one primarily of vitamin D

required per unit weight increase by prophylactic experiment, and this has no immediate relation either to the processes of egg production, maintenance of body weight, successful reproduction and breeding, or parental rearing of the young, since these attributes are functions of the adult fowl. The results of the writers apply, therefore, to the complex process of organic growth during an early stage of the bird's life, and they are based chiefly upon the increase in weight to four weeks of age, when this weight increase is characterized by satisfactory rates of growth and adequate skeletal calcification. These data lend confirmation to the experiments of others mentioned above, which demonstrate that the efficiency of antirachitic substances evaluated in rat units is not equally effective for rats and chicks, especially when the vitamin of irradiated substances is fed comparatively to the two species. It seems clear that, relatively, the rat's requirement is much less than that of the chick for the irradiated form of the vitamin, or else other factors of an heretofore unknown nature operate effectively to cause less efficient utilization by chicks of most irradiated substances with irradiated cholesterol as possibly the only exception.

The results obtained from Groups 23-24 confirm the conclusions of Waddell (7) with respect to the irradiation of cholesterol and the production of calcifying properties by chicks comparable to that shown by rats. This is especially significant in the light of previous work done by the writers, which demonstrated that an equal number of units of this vitamin from cod-liver oil in ration of 100 grams is quite sufficient to produce satisfactory growth and normal calcification in the bones of chicks subsisting upon the A.O.A.C. diet under conditions quite similar to this experiment.

In all bioassays herein reported, the error introduced appears unusually low for this type of work. The importance of standardization and improvement in details of the method to a point where it compares favorably in accuracy with any of the rat assays known heretofore is emphasized and is evident from these experiments. To a discussion of the relative importance of the A.O.A.C. and rat methods of bioassay may be added consideration regarding the manner of common expression of vitamin potency. When expressed in terms of units, such terms are subject to an appreciable error, apart from that inherent in biological feeding experiments. Unless a prohibitive and wasteful number of animals are used, perhaps the better method of expressing vitamin potency for the appearance of accuracy is by means of a definite range of units. For example, to consider a product with a potency of approximately  $100 \pm 10$  units per gram determined by accurate methods of rat bioassays, the substance may more properly be said to have between 90 and 110 units.

Further studies are planned in this laboratory to determine the magnitude and possible direction of error that may exist in the A.O.A.C. method of bioassay. It appears from these preliminary observations that the

errors are no larger by this method than are those evident in rat bioassays.

### SUMMARY

In several experiments with young chicks on which the bone ash and calcium, inorganic phosphorus and magnesium contents of the blood plasma had been determined it appears that:

(1) Irradiated cholesterol is equal to the U. S. Pharmacopoeia reference cod-liver oil in promoting satisfactory growth and bone calcification when 28 U.S.P. units of vitamin D from each substance per hundred grams A.O.A.C. ration per chick are fed for four weeks.

(2) The minerals of blood plasma are a poor and unreliable criterion (in comparison with the ash content of the bones) in interpreting the amount of antirachitic protection afforded chicks by several vitamin-D substances.

(3) Irradiated ergosterol or irradiated yeast even at 50× the vitamin activity of irradiated cholesterol or cod-liver oil fails to produce a normally calcified bone.

(4) When the same amount of vitamin D from both irradiated ergosterol and irradiated yeast is given to chicks, no superiority of one to the other for the production of a normal bone is apparent.

(5) Irradiation for 20 minutes of the A.O.A.C. diet supplemented with corn oil produces a definite calcifying effect in the bones of white Leghorns four weeks old, but such calcification under the conditions employed is slightly below normal.

(6) A newer appreciation of the accuracy of the A.O.A.C. method of vitamin-D assay may be obtained by further consideration and study of the data herein reported.

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## STUDIES RELATIVE TO THE ESTIMATION OF VITAMIN D

### VII. EFFECT OF AGE, SEX, SIZE, AND CALCIFICATION IN YOUNG CHICKS ON ACCURACY OF PREVENTIVE BIOASSAY

By LAWRENCE L. LACHAT (Feed Laboratory, Department of Agriculture, Dairy and Food, St. Paul, Minn.)

Recently Bills and associates (1) statistically estimated the error in line test of rats by curative technic, the method employed for interpreting results being one not particularly useful for comparative purposes. Moreover, the errors obtained by extrapolation of the data do not agree satisfactorily with those for probable error of the bioassays considered. Since these investigators did not explain the differences existing between their computations, the results reported rest on rather tenuous evidence and appear only equivocally significant. Subsequently, Morgan (2) critically examined the source of errors in rat bioassay when line test procedure was used and described a method to determine the degree of healing by measuring the area of new calcification shown by magnified camera-lucida drawings of bone sections. Morgan suggests the chief source of errors to be variable response of litter mates to a given dose of vitamin, and concludes the influence of sex and weight variables to be negligible.

When the curative type of rat vitamin test used by these workers was considered, subjective methods, well illustrated by line test or its modifications, have not been demonstrated to apply specifically for interpreting results of chick bioassay by preventive (prophylactic) technic. Objective methods of substantially greater accuracy have been largely used with this species; as an example, the utility of the bone ash procedure has recently been critically evaluated by several different authorities, St. John and associates (3), Bethke and Record (4), Harshaw *et al.* (5) and Lachat (6).

Despite the satisfactory adaptability of fowls for determining vitamin content, as revealed by many research studies, no thorough-going investigations have been published, so far as the writer is aware, to show that such factors as sex, age, or weight characteristic of the species, may fundamentally influence a prophylactic bioassay. In order to note the effect of these characteristics upon the chick during its early life, and to ascertain the practical application of such data to the A.O.A.C. method of bioassay, the present experiments are described in detail. The task has been accomplished by classifying material into definite groups, based on integral age, sex, size (body weight) and calcification (bone ash content) variables, and subjecting the data to statistical treatment.

Day-old, single-comb, white Leghorns, obtained commercially and housed in electrically-heated battery brooders equipped with raised wire grids, had access to distilled water and the A.O.A.C. vitamin-D deficient ration. Each bird was given all it would consume, the sole variable of the



ration being the kind and amount of vitamin D supplied. Irradiated yeast or 1 per cent of corn oil containing the various oil additions given in Part V of this series of papers, *This Journal*, 19, 628 (1936), supplemented the diet unless otherwise indicated below:

| LOT NO. | SUPPLEMENT ADDED TO A.O.A.C. RATION | U.S.P. VITAMIN-D UNITS PER<br>100 G. RATION |
|---------|-------------------------------------|---|
|         | <i>per cent</i>                     |   |
| 1       | 1.00 corn oil                       | 0.0   |
| 2       | 0.50 low vitamin-D cod-liver oil    | ?   |
| 3       | 0.50 sardine (pilchard) oil         | ?   |
| 4       | 0.13 concentrated cod-liver oil     | ?   |
| 5       | 0.50 high vitamin-D cod-liver oil   | ?   |
| 6       | Same as lot 1, ration irradiated    | ?   |
| 7       | 0.28 irradiated ergosterol          | 703   |
| 8       | 0.56 irradiated ergosterol          | 1406  |
| 9       | 0.25 irradiated yeast               | 630-765                                     |
| 10      | 0.50 irradiated yeast               | 1260-1530                                   |
| 11      | 0.28 cod-liver oil                  | 24  |
| 12      | 0.28 irradiated cholesterol         | 28  |

In the examination of the data statistically the pens were treated separately, each tabulated lot number consisting of two pens of 50 birds each. Preventive technic according to the A.O.A.C. method was used throughout, sex being determined prior to sacrifice or during post-mortem examination of the birds. Average weights of the various pens were computed when the birds were hatched, and thereafter individual weights were recorded weekly. If pathological abnormalities existed during the experiment, these were recorded and the bird discarded. At time of hatch or one, two, three, or four weeks of age, representative birds were killed by breaking the neck, and subsequently the left tibia was ashed by standard procedure (6).

#### SEX DIFFERENCES IN CALCIFICATION

It has been demonstrated that chicks fed adequate amounts of cod-liver oil display sex differences in calcification, the females producing a higher bone ash than do males, Holmes *et al.* (7) and Schroeder (8), a demonstration that unfortunately did not measure the difference between chicks having low ash content deprived partially or wholly of vitamin D. In this connection subsequent study, Lachat (9) and Lachat and Halvorson (10), suggests that the normal difference expected in birds fed adequately may not be evident with decreasing amounts of vitamin. In fact, these investigators found that birds deprived entirely of vitamin D reverse the normal behavior, the males (4 weeks of age) having better calcification than females developing similarly on the same diet. On the ground of this and other observations, the effect of age and the presence or absence of vitamin D upon sex differences in calcification assumes great importance. The relationship during an early stage of the chick's life between

TABLE 1.—Statistical comparison of sex differences in calcification of white Leghorn chicks at various ages that received different forms of vitamin D

| LOT NO.              | SUPPLEMENT ADDED TO A.O.A.C. RATION | MALES |                 |     | FEMALES  |                     |          | MEAN DIFF. | Pd       | per cent |       | Ca   | per cent |  | O <sub>2</sub> -P RATIO |
|----------------------|-------------------------------------|-------|-----------------|-----|----------|---------------------|----------|------------|----------|----------|-------|------|----------|--|-------------------------|
|                      |                                     | NO.   | BONE ASH        | NO. | BONE ASH | NO.                 | BONE ASH |            |          |          |       |      |          |  |                         |
|                      |                                     |       | per cent        |     |          | per cent            |          |            |          |          |       |      |          |  |                         |
|                      |                                     |       | <i>Day old</i>  |     |          | <i>One week old</i> |          |            |          |          |       |      |          |  |                         |
| 5-8-35 <sup>a</sup>  |                                     | 5     | 34.69           | 7   | 34.19    |                     |          | 0.50       | 0.5-0.4  |          |       |      |          |  |                         |
| 5-14-35 <sup>a</sup> |                                     | 3     | 32.94           | 9   | 34.23    |                     |          | -1.29      | 0.3-0.2  |          |       |      |          |  |                         |
| 5-28-35 <sup>a</sup> |                                     | 5     | 33.65           | 7   | 34.95    |                     |          | -1.30      | 0.3-0.2  |          |       |      |          |  |                         |
| 6-11-35 <sup>a</sup> |                                     | 7     | 34.78           | 6   | 35.11    |                     |          | -0.33      | 0.7-0.6  |          |       |      |          |  |                         |
| 6-18-35 <sup>a</sup> |                                     | 7     | 33.91           | 7   | 35.16    |                     |          | -1.25      | 0.1-0.05 |          |       |      |          |  |                         |
| Total                |                                     | 27    | 34.12           | 36  | 34.69    |                     |          | -0.57      | 0.2-0.1  |          |       |      |          |  |                         |
|                      |                                     |       | <i>per cent</i> |     |          | <i>per cent</i>     |          |            |          |          |       |      |          |  |                         |
| 1                    | 1.00 corn oil                       | 5     | 34.54           | 7   | 35.44    |                     |          | -0.90      | 0.6-0.5  | 0.83°    | 0.69° | 1.20 |          |  |                         |
| 1                    | " "                                 | 9     | 37.19           | 3   | 36.35    |                     |          | 0.84       | 0.8-0.7  |          |       |      |          |  |                         |
| 2                    | 0.50 low vit. D cod-liver oil       | 6     | 31.95           | 6   | 33.42    |                     |          | -1.47      | 0.2-0.1  | 0.83°    | 0.68° | 1.22 |          |  |                         |
| 2                    | " " " "                             | 6     | 34.57           | 5   | 34.09    |                     |          | 0.48       | 0.7-0.6  |          |       |      |          |  |                         |
| 11                   | 0.28 cod-liver oil                  | 7     | 36.55           | 5   | 38.47    |                     |          | -1.92      | <0.01°   | 0.84°    | 0.70° | 1.20 |          |  |                         |
| 11                   | " " " "                             | 5     | 36.65           | 7   | 37.87    |                     |          | -1.22      | 0.2-0.1  |          |       |      |          |  |                         |
| 7                    | 0.28 irr. ergosterol                | 7     | 35.24           | 5   | 37.57    |                     |          | -2.33      | 0.2-0.1  | 0.81°    | 0.70° | 1.16 |          |  |                         |
| 7                    | " " " "                             | 9     | 38.56           | 4   | 39.10    |                     |          | -0.54      | 0.8-0.7  |          |       |      |          |  |                         |
| 8                    | 0.56 " " "                          | 6     | 37.59           | 6   | 39.58    |                     |          | -1.99      | 0.2-0.1  | 0.83°    | 0.70° | 1.19 |          |  |                         |
| 8                    | " " " "                             | 3     | 39.60           | 3   | 39.62    |                     |          | -0.02      | >0.9     |          |       |      |          |  |                         |
| 9                    | 0.25 irr. yeast                     | 3     | 35.30           | 9   | 35.92    |                     |          | -0.62      | 0.8-0.7  | 0.82°    | 0.69° | 1.19 |          |  |                         |
| 9                    | " " " "                             | 5     | 36.89           | 7   | 37.65    |                     |          | -0.76      | 0.7-0.6  |          |       |      |          |  |                         |
| 10                   | 0.50 " " "                          | 4     | 39.54           | 8   | 38.58    |                     |          | 0.96       | 0.5-0.4  | 0.83°    | 0.71° | 1.17 |          |  |                         |
| 10                   | " " " "                             | 4     | 40.01           | 8   | 39.13    |                     |          | 0.88       | 0.5-0.4  |          |       |      |          |  |                         |
| 6                    | 1.00 corn oil, ra. irr.             | 8     | 39.00           | 4   | 39.94    |                     |          | -0.94      | 0.4-0.3  | 0.84°    | 0.68° | 1.24 |          |  |                         |
| 6                    | " " " "                             | 5     | 39.96           | 7   | 39.80    |                     |          | 0.16       | 0.8-0.7  |          |       |      |          |  |                         |
| 3                    | 0.50 sard. (pilchard) oil           | 6     | 35.38           | 5   | 37.26    |                     |          | -1.88      | 0.1-0.05 | 0.85°    | 0.68° | 1.25 |          |  |                         |
| 3                    | " " " "                             | 8     | 37.04           | 4   | 36.36    |                     |          | 0.68       | 0.6-0.5  |          |       |      |          |  |                         |
| 4                    | 0.13 con. cod-liver oil             | 4     | 37.14           | 7   | 35.97    |                     |          | 1.17       | 0.6-0.5  | 0.84°    | 0.68° | 1.24 |          |  |                         |
| 4                    | " " " "                             | 6     | 35.98           | 6   | 37.75    |                     |          | -1.77      | 0.2-0.1  |          |       |      |          |  |                         |
| 5                    | 0.50 high vit. D cod-liver oil      | 5     | 38.13           | 7   | 37.29    |                     |          | 0.84       | 0.6-0.5  | 0.86°    | 0.68° | 1.26 |          |  |                         |
| 5                    | " " " "                             | 4     | 38.06           | 7   | 38.88    |                     |          | -0.82      | 0.7-0.6  |          |       |      |          |  |                         |
| 12                   | 0.28 irr. cholesterol               | 5     | 38.55           | 7   | 37.20    |                     |          | 1.35       | 0.3-0.2  | 0.84°    | 0.71° | 1.18 |          |  |                         |
| 12                   | " " " "                             | 6     | 38.73           | 6   | 40.38    |                     |          | -1.65      | 0.3-0.2  |          |       |      |          |  |                         |
| Total                |                                     | 141   | 37.18           | 143 | 37.61    |                     |          | -0.43      | 0.2-0.1  |          |       |      |          |  |                         |

TABLE 1.—(Continued)

| LOT NO. | SUPPLEMENT ADDED TO A.O.A.C. RATION | MALES |                      | FEMALES |          | MEAN DIFF. | Pd         | RATION MIX. ANAL. |          | Ca-P RATIO |
|---------|-------------------------------------|-------|----------------------|---------|----------|------------|------------|-------------------|----------|------------|
|         |                                     | NO.   | BONE ASH             | NO.     | BONE ASH |            |            | Ca                | P        |            |
|         |                                     |       | per cent             |         | per cent | per cent   |            | per cent          | per cent |            |
|         |                                     |       | <i>Two weeks old</i> |         |          |            |            |                   |          |            |
| 1       | 1.00 corn oil                       | 7     | 33.73                | 5       | 36.05    | -2.32      | 0.5-0.4    |                   |          |            |
| 1       | " "                                 | 6     | 35.54                | 6       | 34.87    | 0.67       | 0.8-0.7    |                   |          |            |
| 2       | 0.50 low vit. D cod-liver oil       | 7     | 32.93                | 4       | 34.56    | -1.63      | 0.5-0.4    |                   |          |            |
| 2       | " "                                 | 6     | 34.28                | 5       | 36.30    | -2.02      | 0.4-0.3    |                   |          |            |
| 11      | 0.28 cod-liver oil                  | 6     | 38.79                | 6       | 35.75    | 3.04       | 0.2-0.1    |                   |          |            |
| 11      | " "                                 | 6     | 37.75                | 6       | 41.17    | -3.42      | 0.2-0.1    |                   |          |            |
| 7       | 0.28 irr. ergosterol                | 4     | 39.91                | 9       | 41.11    | -1.20      | 0.4-0.3    |                   |          |            |
| 7       | " "                                 | 6     | 40.63                | 7       | 41.33    | -0.70      | 0.8-0.7    |                   |          |            |
| 8       | 0.56 " "                            | 7     | 40.92                | 5       | 43.51    | -2.59      | 0.2-0.1    |                   |          |            |
| 8       | " "                                 | 8     | 42.83                | 4       | 41.90    | 0.93       | 0.4-0.3    |                   |          |            |
| 9       | 0.25 irr. yeast                     | 9     | 40.60                | 2       | 40.91    | -0.31      |            |                   |          |            |
| 9       | " "                                 | 7     | 40.88                | 4       | 43.46    | -2.58      | <0.01*     |                   |          |            |
| 10      | 0.50 " "                            | 7     | 42.27                | 4       | 43.23    | -0.96      | 0.3-0.2    |                   |          |            |
| 10      | " "                                 | 3     | 42.81                | 9       | 42.80    | 0.01       | >0.9       |                   |          |            |
| 6       | 1.00 corn oil, ra. irr.             | 7     | 42.59                | 5       | 42.29    | 0.30       | 0.8-0.7    |                   |          |            |
| 6       | " "                                 | 6     | 42.61                | 6       | 42.49    | 0.12       | 0.9-0.8    |                   |          |            |
| 3       | 0.50 sard. (pilchard) oil           | 5     | 38.91                | 6       | 38.76    | 0.15       | 0.9-0.8    |                   |          |            |
| 3       | " "                                 | 3     | 37.76                | 8       | 42.52    | -4.76      | <0.01*     |                   |          |            |
| 4       | 0.13 con. cod-liver oil             | 5     | 42.01                | 7       | 40.83    | 1.18       | 0.5-0.4    |                   |          |            |
| 4       | " "                                 | 2     | 41.67                | 8       | 41.90    | -0.23      |            |                   |          |            |
| 5       | 0.50 high vit. D cod-liver oil      | 4     | 37.72                | 7       | 41.97    | -4.25      | 0.2-0.1    |                   |          |            |
| 5       | " "                                 | 5     | 40.30                | 7       | 42.91    | -2.61      | 0.1-0.05   |                   |          |            |
| 12      | 0.28 irr. cholesterol               | 7     | 43.80                | 5       | 44.06    | -0.26      | 0.9-0.8    |                   |          |            |
| 12      | " "                                 | 8     | 43.16                | 4       | 45.66    | -2.50      | 0.2-0.1    |                   |          |            |
| Total   |                                     | 141   | 39.80                | 139     | 40.91    | -1.11      | 0.05-0.02* |                   |          |            |

|       |                                | <i>Three weeks old</i> |       |     |       |       |            |  |  |
|-------|--------------------------------|------------------------|-------|-----|-------|-------|------------|--|--|
|       |                                | 5                      | 31.94 | 7   | 28.55 | 3.39  | 0.2-0.1    |  |  |
| 1     | 1.00 corn oil                  | 6                      | 31.68 | 5   | 28.93 | 2.75  | 0.2-0.1    |  |  |
| 1     | " "                            | 4                      | 34.21 | 7   | 30.47 | 3.74  | 0.1-0.05   |  |  |
| 2     | 0.50 low vit. D cod-liver oil  | 8                      | 31.87 | 5   | 31.94 | -0.07 | >0.9       |  |  |
| 2     | " " " "                        | 4                      | 38.36 | 8   | 38.41 | -0.05 | >0.9       |  |  |
| 11    | 0.28 cod-liver oil             | 8                      | 40.22 | 4   | 37.62 | 2.60  | 0.3-0.2    |  |  |
| 11    | " " " "                        | 7                      | 43.49 | 6   | 37.67 | 5.82  | 0.1-0.05   |  |  |
| 7     | 0.28 irr. ergosterol           | 4                      | 42.98 | 8   | 42.09 | 0.89  | 0.7        |  |  |
| 7     | " " " "                        | 3                      | 43.73 | 9   | 43.25 | 0.48  | 0.8-0.7    |  |  |
| 8     | 0.56 " " "                     | 9                      | 44.28 | 4   | 44.36 | -0.08 | >0.9       |  |  |
| 8     | " " " "                        | 4                      | 41.14 | 8   | 42.20 | -1.06 | 0.7-0.6    |  |  |
| 9     | 0.25 irr. yeast                | 5                      | 43.12 | 7   | 43.75 | -0.63 | 0.6-0.5    |  |  |
| 9     | " " " "                        | 6                      | 42.82 | 6   | 43.34 | -0.52 | 0.8-0.7    |  |  |
| 10    | 0.50 " " "                     | 10                     | 43.21 | 2   | 44.08 | -0.87 |            |  |  |
| 6     | 1.00 corn oil, ra. irr.        | 5                      | 43.19 | 7   | 45.09 | -1.90 | <0.01*     |  |  |
| 6     | " " " "                        | 6                      | 44.34 | 6   | 44.41 | -0.07 | >0.9       |  |  |
| 3     | 0.50 sard. (pilchard) oil      | 5                      | 43.19 | 7   | 42.90 | 0.29  | 0.9        |  |  |
| 3     | " " " "                        | 4                      | 43.50 | 7   | 43.34 | 0.16  | >0.9       |  |  |
| 4     | 0.13 con. cod-liver oil        | 8                      | 43.80 | 3   | 45.21 | -1.41 | 0.2-0.1    |  |  |
| 4     | " " " "                        | 6                      | 44.31 | 5   | 44.75 | -0.44 | 0.7-0.6    |  |  |
| 5     | 0.50 high vit. D cod-liver oil | 9                      | 43.76 | 2   | 43.95 | -0.19 |            |  |  |
| 5     | " " " "                        | 7                      | 44.91 | 5   | 44.51 | 0.40  | 0.3-0.2    |  |  |
| 12    | 0.28 irr. cholesterol          | 3                      | 44.10 | 9   | 46.21 | -2.11 | <0.01*     |  |  |
| 12    | " " " "                        | 7                      | 45.46 | 6   | 46.36 | -0.90 | 0.05-0.02* |  |  |
| Total |                                | 143                    | 41.53 | 143 | 40.88 | 0.65  | 0.4-0.3    |  |  |

TABLE 1.—(Continued)

| LOT NO. | SUPPLEMENT ADDED TO A.O.A.C. RATION | MALES |       |      | FEMALES               |     |      | MEAN DIFF. | Pd       | RATON MUL. ANAL. |   |            |
|---------|-------------------------------------|-------|-------|------|-----------------------|-----|------|------------|----------|------------------|---|------------|
|         |                                     | NO.   | AGE   | BONE | NO.                   | AGE | BONE |            |          | Cs               | P | Ca-P RATIO |
|         |                                     |       |       |      |                       |     |      | per cent   |          | per cent         |   | per cent   |
|         |                                     |       |       |      | <i>Four weeks old</i> |     |      | per cent   |          |                  |   |            |
| 1       | 1.00 corn oil                       | 7     | 30.48 | 6    | 29.66                 |     |      | 0.82       | 0.5-0.4  |                  |   |            |
| 1       | " "                                 | 6     | 30.37 | 8    | 30.23                 |     |      | 0.14       | >0.9     |                  |   |            |
| 2       | 0.50 low vit. D cod-liver oil       | 5     | 32.65 | 6    | 29.52                 |     |      | 3.13       | 0.2-0.1  |                  |   |            |
| 2       | " " " "                             | 6     | 30.79 | 6    | 31.09                 |     |      | -0.30      | 0.8-0.7  |                  |   |            |
| 11      | 0.28 cod-liver oil                  | 4     | 40.54 | 8    | 36.94                 |     |      | 3.60       | 0.1-0.05 |                  |   |            |
| 11      | " " " "                             | 4     | 36.31 | 8    | 39.25                 |     |      | -2.94      | 0.4-0.3  |                  |   |            |
| 7       | " irr. ergosterol                   | 5     | 39.40 | 8    | 41.50                 |     |      | -2.10      | 0.3-0.2  |                  |   |            |
| 7       | " " " "                             | 3     | 41.19 | 9    | 41.43                 |     |      | -0.24      | >0.9     |                  |   |            |
| 8       | 0.56 " "                            | 3     | 43.18 | 8    | 44.58                 |     |      | -1.40      | 0.2-0.1  |                  |   |            |
| 8       | " " " "                             | 11    | 44.20 | 1    | 44.88                 |     |      | -0.68      |          |                  |   |            |
| 9       | 0.25 irr. yeast                     | 6     | 43.33 | 5    | 43.48                 |     |      | -0.15      | 0.9-0.8  |                  |   |            |
| 9       | " " " "                             | 7     | 43.30 | 4    | 45.62                 |     |      | -2.32      | 0.1-0.05 |                  |   |            |
| 10      | 0.50 " "                            | 5     | 43.25 | 6    | 44.54                 |     |      | -1.29      | 0.4-0.3  |                  |   |            |
| 10      | " " " "                             | 5     | 44.21 | 6    | 44.67                 |     |      | -0.46      | 0.5-0.4  |                  |   |            |
| 6       | 1.00 corn oil, ra. irr.             | 5     | 42.31 | 6    | 44.65                 |     |      | -2.34      | <0.01*   |                  |   |            |
| 6       | " " " "                             | 5     | 43.21 | 7    | 44.57                 |     |      | -1.36      | 0.3-0.2  |                  |   |            |
| 3       | 0.50 sard. (pilchard) oil           | 7     | 44.13 | 4    | 45.87                 |     |      | -1.74      | 0.3-0.2  |                  |   |            |
| 3       | " " " "                             | 6     | 45.25 | 5    | 44.34                 |     |      | 0.91       | 0.7-0.6  |                  |   |            |
| 4       | 0.13 con. cod-liver oil             | 7     | 44.41 | 4    | 45.75                 |     |      | -1.34      | 0.2-0.1  |                  |   |            |
| 4       | " " " "                             | 5     | 45.91 | 6    | 45.02                 |     |      | 0.89       | 0.3-0.2  |                  |   |            |
| 5       | 0.50 high vit. D cod-liver oil      | 3     | 45.84 | 7    | 45.86                 |     |      | -0.02      | >0.9     |                  |   |            |
| 5       | " " " "                             | 3     | 44.88 | 9    | 46.75                 |     |      | -1.87      | <0.01*   |                  |   |            |
| 12      | 0.28 irr. cholesterol               | 7     | 46.26 | 5    | 46.24                 |     |      | 0.02       | >0.9     |                  |   |            |
| 12      | " " " "                             | 7     | 46.17 | 5    | 46.60                 |     |      | -0.43      | 0.4-0.3  |                  |   |            |
| Total   |                                     | 132   | 41.33 | 147  | 41.35                 |     |      | -0.02      | >0.9     |                  |   |            |

*Selected Bioassays (1934-35)*

| None   | 4  | 28.23 | 9  | 27.82 | 0.41  | 0.8-0.7    | 0.89 | 0.68 | 1.31 |
|--|----|-------|----|-------|-------|------------|------|------|------|
| 1.00 corn oil                                      | 10 | 29.06 | 5  | 29.75 | -0.69 | 0.6-0.5    | 0.92 | 0.67 | 1.37 |
| 2.63 charcoal <sup>b</sup>                         | 6  | 29.18 | 7  | 28.40 | 0.78  | 0.2-0.1    | 0.85 | 0.66 | 1.29 |
| 0.50 com. cod-liver oil (CLO-103)                  | 5  | 29.60 | 7  | 30.12 | -0.52 | 0.7-0.6    | 0.83 | 0.68 | 1.22 |
| 10.00 irr. yeast (CLO-59)                          | 4  | 29.90 | 6  | 29.41 | 0.49  | 0.8-0.7    | 0.79 | 0.67 | 1.18 |
| 1.00 corn oil                                      | 8  | 30.23 | 9  | 29.92 | 0.31  | 0.8-0.7    | 0.86 | 0.72 | 1.19 |
| 5.00 irr. yeast (CLO-59)                           | 4  | 30.29 | 7  | 29.15 | 1.14  | 0.2-0.1    | 0.84 | 0.68 | 1.24 |
| 0.06 U.S.P. ref. cod-liver oil                     | 5  | 30.61 | 8  | 30.07 | 0.54  | 0.7-0.6    | 0.90 | 0.70 | 1.29 |
| 0.25 com. cod-liver oil (CLO-63)                   | 3  | 30.91 | 7  | 33.95 | -3.04 | 0.3-0.2    | 0.84 | 0.69 | 1.22 |
| 0.06 com. cod-liver oil (CLO-55)                   | 3  | 30.97 | 7  | 29.35 | 1.62  | 0.4-0.3    | 0.83 | 0.67 | 1.24 |
| 0.38 com. cod-liver oil + 1.13% char. <sup>b</sup> | 15 | 31.18 | 13 | 32.68 | -1.50 | 0.1-0.05   | 0.94 | 0.66 | 1.42 |
| 0.13 con. cod-liver oil (CLO-72)                   | 4  | 31.73 | 8  | 34.36 | -2.63 | 0.1-0.05   | 0.84 | 0.65 | 1.29 |
| 0.50 com. cod-liver oil (CLO-105)                  | 5  | 31.76 | 7  | 31.37 | 0.39  | 0.8-0.7    | 0.79 | 0.65 | 1.22 |
| 1.00 corn oil                                      | 6  | 32.22 | 10 | 32.19 | 0.03  | >0.9       | 0.81 | 0.65 | 1.25 |
| 0.25 com. cod-liver oil (CLO-64)                   | 4  | 32.40 | 6  | 34.19 | -1.79 | 0.4-0.3    | 0.84 | 0.73 | 1.15 |
| 0.38 com. cod-liver oil                            | 11 | 32.76 | 18 | 31.93 | 0.83  | 0.3-0.2    | 0.91 | 0.69 | 1.32 |
| 0.25 sard. (pilchard) oil (CLO-95)                 | 4  | 32.91 | 7  | 33.37 | -0.46 | 0.8-0.7    | 0.85 | 0.65 | 1.31 |
| 0.06 con. cod-liver oil (CLO-109)                  | 7  | 33.30 | 4  | 34.51 | -1.21 | 0.4-0.3    | 0.83 | 0.66 | 1.26 |
| 0.50 com. cod-liver oil (CLO-96)                   | 3  | 33.37 | 8  | 34.37 | -1.00 | 0.8-0.7    | 0.79 | 0.71 | 1.11 |
| 0.13 con. cod-liver oil (CLO-65)                   | 6  | 33.39 | 7  | 30.29 | 3.10  | 0.05-0.02* | 0.89 | 0.65 | 1.37 |
| 0.50 com. cod-liver oil (CLO-106)                  | 7  | 33.44 | 6  | 34.41 | -0.97 | 0.5-0.4    | 0.79 | 0.65 | 1.22 |
| 10.00 cod-liver meal (CLO-88)                      | 3  | 33.52 | 5  | 32.76 | 0.76  | 0.4-0.3    | 0.78 | 0.67 | 1.16 |
| 1.00 corn oil                                      | 14 | 34.03 | 5  | 34.26 | -0.23 | 0.9-0.8    | 0.84 | 0.65 | 1.29 |
| 0.13 U.S.P. ref. cod-liver oil                     | 7  | 34.19 | 6  | 34.15 | 0.04  | >0.9       | 0.92 | 0.68 | 1.35 |

TABLE 1.—(Continued)

| LOT NO.   | SUPPLEMENT ADDED TO A.O.A.C. RATION | MALES |          | FEMALES |          | MEAN DIFF. | Pd       | RATON MED. ANAL. |          | C+P RATIO |
|---|-------------------------------------|-------|----------|---------|----------|------------|----------|------------------|----------|-----------|
|   |                                     | NO.   | BONE ASH | NO.     | BONE ASH |            |          | Ca               | P        |           |
|   |                                     |       | per cent |         | per cent | per cent   |          | per cent         | per cent |           |
| 0.13 con. cod-liver oil (CLO-55)                            |                                     | 5     | 34.77    | 8       | 37.55    | -2.78      | 0.3-0.2  | 0.88             | 0.67     | 1.31      |
| 0.50 com. cod-liver oil (CLO-90)                            |                                     | 4     | 35.59    | 8       | 34.44    | 1.15       | 0.7-0.6  | 0.81             | 0.69     | 1.17      |
| 5.00 cod-liver meal (CLO-88)                                |                                     | 3     | 35.65    | 7       | 33.77    | 1.88       | 0.3-0.2  | 0.79             | 0.68     | 1.16      |
| 0.50 com. cod-liver oil (CLO-63)                            |                                     | 5     | 35.95    | 6       | 39.32    | -3.37      | 0.3-0.2  | 0.82             | 0.66     | 1.24      |
| 0.13 con. cod-liver oil (CLO-67)                            |                                     | 7     | 36.68    | 5       | 38.67    | -1.99      | 0.5-0.4  | 0.86             | 0.67     | 1.28      |
| 0.63 com. cod-liver oil + 1.88% char. <sup>b</sup> (CLO-61) |                                     | 13    | 37.44    | 13      | 35.79    | 1.65       | 0.3-0.2  | 0.91             | 0.65     | 1.40      |
| 0.25 sard. (pilchard) oil (CLO-61)                          |                                     | 5     | 37.54    | 7       | 37.65    | -0.11      | >0.9     | 0.82             | 0.65     | 1.26      |
| 0.50 com. cod-liver oil (CLO-74)                            |                                     | 7     | 37.64    | 4       | 35.94    | 1.70       | 0.5-0.4  | 0.85             | 0.65     | 1.31      |
| 0.63 com. cod-liver oil                                     |                                     | 14    | 37.94    | 13      | 36.10    | 1.84       | 0.3-0.2  | 0.89             | 0.68     | 1.31      |
| 0.25 com. cod-liver oil (CLO-108)                           |                                     | 5     | 38.18    | 7       | 35.80    | 2.38       | 0.3-0.2  | 0.81             | 0.65     | 1.25      |
| 1.00 com. cod-liver oil (CLO-90)                            |                                     | 10    | 38.22    | 3       | 41.20    | -2.98      | 0.4-0.3  | 0.80             | 0.66     | 1.21      |
| 0.50 sard. (pilchard) oil (CLO-57)                          |                                     | 3     | 38.28    | 9       | 37.85    | 0.43       | 0.9-0.8  | 0.92             | 0.70     | 1.31      |
| 0.25 sard. (pilchard) oil (CLO-91)                          |                                     | 4     | 38.87    | 6       | 38.01    | 0.86       | 0.8-0.7  | 0.79             | 0.67     | 1.18      |
| 0.50 com. cod-liver oil (CLO-56)                            |                                     | 3     | 39.50    | 7       | 37.96    | 1.54       | 0.6-0.5  | 0.83             | 0.67     | 1.24      |
| 0.13 con. cod-liver oil (CLO-87)                            |                                     | 3     | 40.40    | 7       | 41.06    | -0.66      | 0.9-0.8  | 0.80             | 0.63     | 1.27      |
| 0.50 sard. (pilchard) oil (CLO-100)                         |                                     | 8     | 40.92    | 3       | 44.10    | -3.18      | 0.4-0.3  | 0.80             | 0.70     | 1.14      |
| 0.50 com. cod-liver oil (CLO-104)                           |                                     | 6     | 41.36    | 6       | 41.71    | -0.35      | 0.9-0.8  | 0.79             | 0.66     | 1.20      |
| 0.06 con. cod-liver oil (CLO-71)                            |                                     | 4     | 41.40    | 6       | 40.40    | 1.00       | 0.8-0.7  | 0.85             | 0.67     | 1.27      |
| 0.19 U.S.P. ref. cod-liver oil                              |                                     | 6     | 41.60    | 7       | 44.26    | -2.66      | 0.2-0.1  | 0.84             | 0.68     | 1.24      |
| 0.50 com. cod-liver oil (CLO-64)                            |                                     | 9     | 41.65    | 3       | 41.04    | 0.61       | 0.9-0.8  | 0.82             | 0.67     | 1.22      |
| 0.25 U.S.P. ref. cod-liver oil                              |                                     | 10    | 41.66    | 3       | 45.55    | -3.89      | 0.2-0.1  | 0.83             | 0.67     | 1.24      |
| 0.25 " " "  |                                     | 4     | 41.80    | 9       | 43.33    | -1.53      | 0.5-0.4  | 0.80             | 0.69     | 1.16      |
| 0.88 com. cod-liver oil                                     |                                     | 11    | 41.82    | 15      | 43.24    | -1.42      | 0.4-0.3  | 0.91             | 0.65     | 1.40      |
| 0.19 U.S.P. ref. cod-liver oil                              |                                     | 6     | 42.30    | 7       | 37.14    | 5.16       | 0.1-0.05 | 0.79             | 0.71     | 1.11      |
| 0.22 " " "  |                                     | 4     | 42.63    | 9       | 41.68    | 0.95       | 0.8-0.7  | 0.78             | 0.70     | 1.11      |
| 0.50 com. cod-liver oil (CLO-97)                            |                                     | 4     | 43.41    | 7       | 44.97    | -1.56      | 0.5-0.4  | 0.85             | 0.64     | 1.33      |
| 0.19 con. cod-liver oil (CLO-84)                            |                                     | 3     | 43.56    | 8       | 43.02    | 0.54       | 0.8-0.7  | 0.82             | 0.67     | 1.22      |





sex differences in calcification and both age and content of vitamin D in the ration has been studied in this laboratory by means of statistical technic, Table 1 summarizing the essential data. It will be observed that the mineral content of the various rations was quite uniform, varying for calcium, 0.77–0.94 per cent, and for phosphorus, 0.63–0.73 per cent. Although bone ash contents are shown by the tabulation when the lots had less than three individuals of either sex per pen, these were not compared statistically, as the validity of conclusions drawn from such data may be seriously impugned. A few specially selected lots have been included for further comparison when birds four weeks old subsisted on the A.O.A.C. ration with various supplements allegedly containing vitamin D.

It will be noted that newly hatched (day-old) birds, considered either as individual pens or together by total for the group, exhibited sex differences in calcification generally favoring the female sex and not statistically significant. Birds one week of age displayed a somewhat smaller sex difference than those newly hatched, and when individually canvassed only one pen revealed a significant difference. It is interesting to note, although difficult to explain, that the second pen of this lot, fed and treated similarly, showed a sex difference like that of the first pen, but not significant statistically.

Birds two weeks of age revealed a statistically significant sex difference, and a higher ash content for females than for males, one pen in each of Lots 9 and 3 showing an analogous difference that was statistically significant. However, opposite pens corresponding to these lots showed ash contents that were not significantly different.

With birds three weeks old one pen (Lot 6) and the pens of Lot 12 showed sex differences in calcification that were significant, and a higher ash content for females than males. Despite these significant differences the total for the group displayed a sex difference in calcification favoring males not statistically significant.

In two pens of birds four weeks old (Lots 6 and 5) the ash content was significantly higher for the female sex. The calcification of both males and females was otherwise remarkably alike, especially when the total of the group is considered. In general, the sex differences in calcification that occurred from time of hatch to four weeks of age reveal the females as having the higher ash content, and these are the only sex differences statistically significant. With the exception of one age group (2 weeks), the sex differences were not positively significant statistically. Also it will be observed that the ash content of all chicks at the various weekly intervals increased with increasing age, but after three weeks very little increase occurred.

When the specially selected lots are included, there is some evidence that feeding amounts of vitamin D that are excessive for the normal re-

quirement produced a pronounced sex difference significant statistically, and this difference always favored the females.

The reversal of the sex difference in calcification as previously shown for birds deprived of vitamin (9)(10) does not appear applicable in this respect to these experiments when birds are two weeks of age or younger, since the sex differences at these ages were consistently higher for females than for males. It is evident, therefore, that age of the chick younger than three weeks, even when the supply of vitamin is deficient, does not cause the phenomenon to appear. In view of the evidence presented, the theory that reversal of the normal sex difference may occur due to early age and

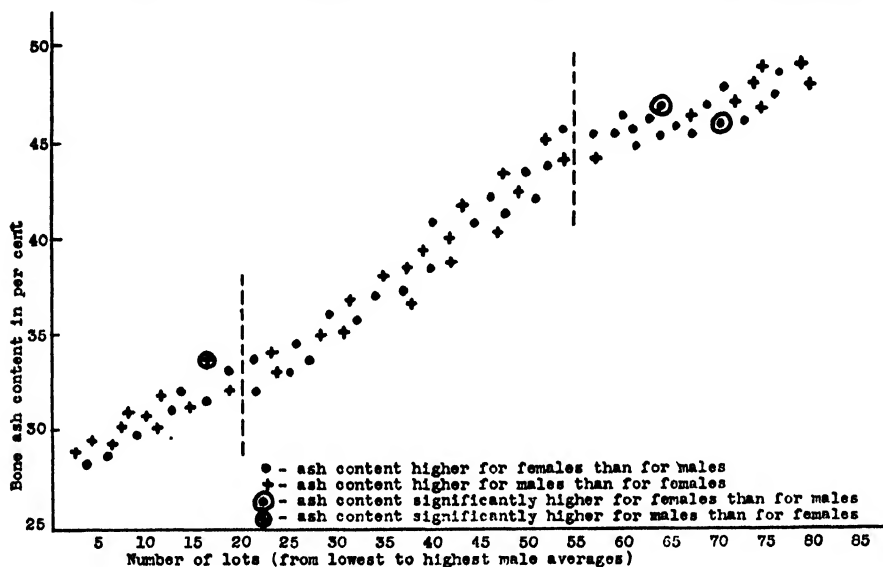


FIG. 1.—Sex differences in calcification of white Leghorns four weeks of age shown comparatively.

be similar to that which occurs for birds 3 or 4 weeks old deprived of vitamin does not receive any support from these experiments. On the other hand these data indicate that deprivation of vitamin D and the resulting retardation of growth associated with an extremely low ash content reverse the normal sex difference in calcification if the birds are 3 or 4 weeks old, and this seems due solely to absence of vitamin during a time when the bone salts may be depleted subnormally.

Fig. 1 presents the sex differences in calcification when chosen from the "selected bioassays" and shows graphically the reversal of the normal sex difference favoring females to one favoring males if the birds are deprived of vitamin four weeks. The three divisions made by the two broken lines clearly confirm the evidence for reversal when it occurs under conditions of greatly retarded growth.

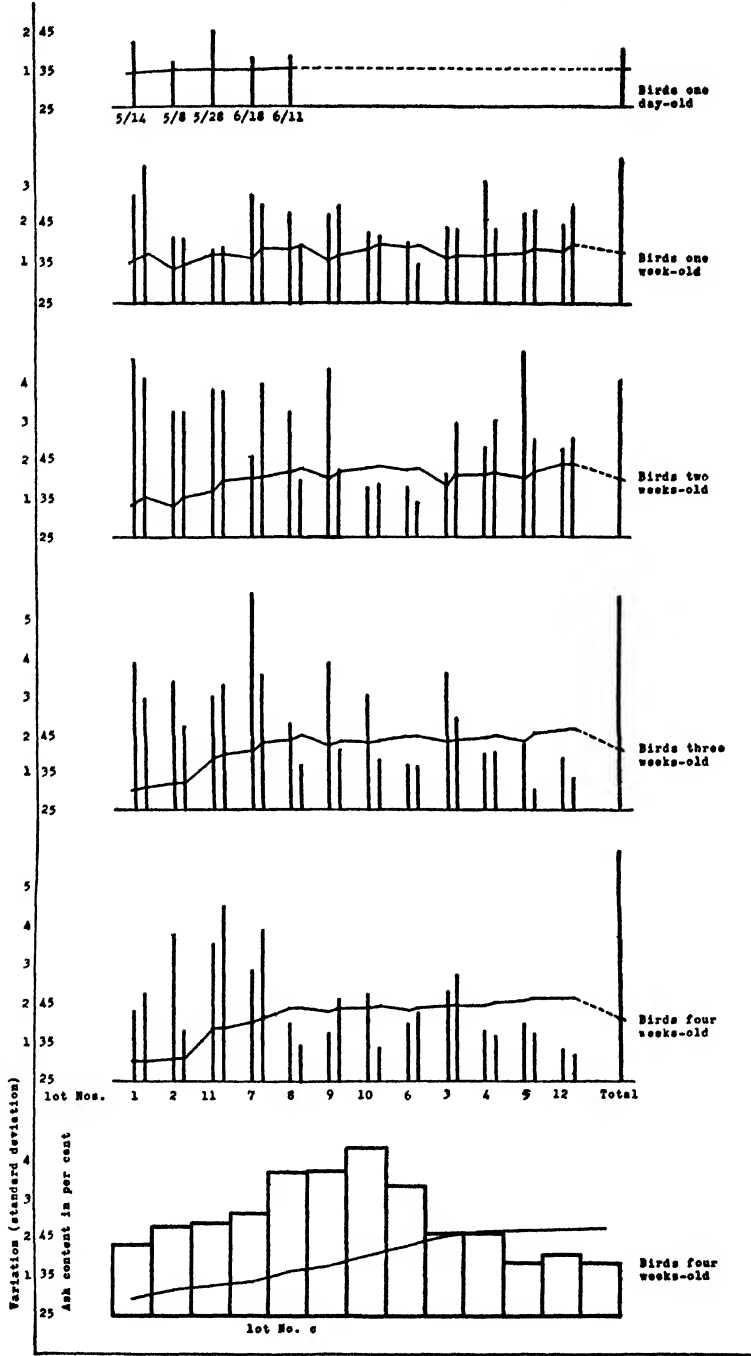


FIG. 2.—Variation in calcification of white Leghorns at various ages that received different forms of vitamin D.



Recapitulating the discussion of data, the weight of evidence convincingly supports the belief that the influence of sex under conditions imposed may be eliminated when average ash contents are computed for interpretative study of results. Accordingly, if the length of test period is shortened by one week, Halvorson and Lachat (11), the error due to this influence may be neglected.

#### SEX DIFFERENCE IN BODY WEIGHTS DURING GROWTH

Table 2 presents the growth records<sup>1</sup> of birds that received different forms of vitamin D supplementing A.O.A.C. ration, and definitely demonstrates that lack of vitamin retards growth when chicks are fed prophylactically, a demonstration that is in accord with the observation that growth is affected when vitamin D is lacking in amounts required for adequate calcification and normal rates of growth in almost direct proportion to the amount of calcification produced. The tabulation reveals the body weights at different ages when various forms of vitamin were fed (a further discussion of comparative weight records for birds at different ages and seasons eating cod-liver oil or other vitamin substance has been given recently by Halvorson and Lachat (12)). Since all birds were started on experiment in less than six weeks the possible effect of season upon growth and calcification was greatly minimized.

Reference to the table will show that the males in general weighed more at all ages than did the females, and that initial weight by sexes was more than doubled, tripled, and quadrupled at 2, 3, and 4 weeks of age, respectively, depending upon the form and supply of vitamin D. Further information relating growth to body weight variables may be had if desired from inspecting the results given by Table 3. There are possible deficiencies other than vitamin D in the nutritive value of the A.O.A.C. ration that caused it to be particularly effective in producing a low bone ash in chicks at an early age. This is shown by the rate of growth, which increased abnormally when specific substances were fed supplementing the ration, notably irradiated yeast and sardine oil. Obviously, some specific property or growth-promoting substance was present in these two materials that produced a better than average rate of growth.

#### ASSOCIATION BETWEEN SIZE AND CALCIFICATION

In view of the marked retardation in growth of chicks caused by lack of vitamin, the relation between size and calcification seems to be a problem of great importance in studying vitamin requirement of chicks. Accordingly, a statistical survey of the data obtained by the writer relating body weight to bone ash content by sexes has been made (Table 3). The results of the correlations computed from weight and ash content at

<sup>1</sup> Growth curves constructed from these data were not representative of weight gains in birds fed vitamin-D substances as previously reported, possibly because (1) the number of birds started in the experiments were decreased by 25% at subsequent weekly intervals, (2) of the nature of the vitamin supplement added, or (3) of reasons that at present remain obscure.

TABLE 3.—Association between calcification and growth by sexes of white Leghorns at various ages that received different forms of vitamin D

| GROUPS          | AGE | NO. OF CHICKS |     | COR. ( $\pm$ S.E.) BFT. BONE ASH AND BODY WT. |                   | STAT. SIG. <sup>b</sup> | DIFF. OF $\bar{x}$ (FISHER, '32) | STAT. SIG. OF DIFF. | AV. BODY WT. (GRAMS) |       | VAR. IN BODY WT. (S.D. $\pm$ S.E.) |                |                |
|-----------------|-----|---------------|-----|---|-------------------|-------------------------|----------------------------------|---------------------|----------------------|-------|------------------------------------|----------------|----------------|
|                 |     | M             | F   | M   | F                 |                         |                                  |                     | M                    | F     | M                                  | F              |                |
| <i>lot nos.</i> |     |               |     |   |                   |                         |                                  |                     |                      |       |                                    |                |                |
| 1 (1-12 inc.)   | 1   | 141           | 143 | 0.366 $\pm$ 0.073                             | 0.314 $\pm$ 0.076 | p                       | p                                | 0.059 $\pm$ 0.120   | n                    | 60.0  | 57.6                               | 9.3 $\pm$ 0.6  | 8.9 $\pm$ 0.5  |
| 2 (1-12 inc.)   | 2   | 141           | 139 | 0.355 $\pm$ 0.074                             | 0.334 $\pm$ 0.076 | p                       | p                                | 0.024 $\pm$ 0.121   | n                    | 93.3  | 92.1                               | 16.7 $\pm$ 1.0 | 15.9 $\pm$ 1.0 |
| 3 (1-12 inc.)   | 3   | 143           | 143 | 0.465 $\pm$ 0.066                             | 0.668 $\pm$ 0.046 | p                       | p                                | -0.304 $\pm$ 0.120  | p                    | 141.1 | 134.7                              | 23.6 $\pm$ 1.4 | 24.2 $\pm$ 1.4 |
| 4 (1-12 inc.)   | 4   | 132           | 147 | 0.736 $\pm$ 0.040                             | 0.810 $\pm$ 0.028 | p                       | p                                | -0.187 $\pm$ 0.121  | n                    | 197.2 | 186.6                              | 5.6 $\pm$ 0.4  | 6.2 $\pm$ 0.4  |
| T <sup>a</sup>  | 4   | 458           | 539 | 0.759 $\pm$ 0.020                             | 0.819 $\pm$ 0.014 | p                       | p                                | -0.160 $\pm$ 0.064  | p                    | 200.1 | 184.2                              |                |                |

<sup>a</sup> Obtained from a former report for comparative purposes.<sup>b</sup> n = negative; p = positive.

the various weekly intervals were positive in every case, the heavier birds producing a higher ash content than the lighter ones. If the correlations had been calculated between *body weight increase* and ash content, they undoubtedly would have been of even greater significance since the initial weights of the birds were somewhat dissimilar (Table 2), averaging from 37 to 42 grams each.

The positive nature of the correlations obtained for both sexes strongly suggests that body weight may be an important criterion to supplement that concerning ash content in interpreting results of bioassay. It is also manifest that the correlation coefficients regularly increased in size with advancing age for both sexes and part of the increase was probably due to the greater body weight attained in both sexes with increasing age, which was also associated with lack of a corresponding increasing variation in ash content. Curiously, none of the groups, excepting the birds three weeks old, showed a statistically significant sex difference between correlations, the correlation in this case being significantly greater for females than for males. This sex difference is evidently related to the one of lot T that is shown for comparative purposes. A definite trend is not apparent from the data relating correlation differences to sex, the males and females having higher correlations than the opposite sex at one and two, and three and four weeks, respectively.

It will be manifest that the results for birds four weeks of age exhibited correlations having a remarkable similarity to each other in either sex, although in the latter instance (group T), the error of the difference of  $z$ , Fisher (13), was small (possibly due to the greater number of birds used in this computation) and a significant sex difference between correlations was observed. Continuing the comparison between the latter two groups further, it will be evident that the average body weights of the respective groups were also very much alike.

Study of the averages and body weight variables shows that variation increased in both sexes until three weeks of age, which was incidentally associated with a corresponding weight increase, and later a sudden well-defined decreasing variability occurred for both sexes that is similar to the same property in Group 1. This decreased variability may possibly be explained on the ground of the selection of birds, the heaviest, lightest, and two of average weight, or multiples thereof of these, being used at weekly intervals, leaving those more uniform in weight for subsequent parts of the experiment. It was not until the fourth week, however, that the method of selection and the resultant incidence of increasing uniformity in weight was observed as shown by standard deviations. It is not surprising that the average weight in each group was greater for males than for females, since the former sex always weighs more at every age on the average, regardless of the supply of vitamin D. In all of these experiments and also in former ones, the writer has not been able to demon-

strate a significant sex difference in weight variability, this property at all ages being characteristic and quite similar for both males and females.

It will be apparent that the data herein reported would not apply to single bioassays employing 10 birds or less, since small numbers limit the validness of the conclusions drawn. It will be noted further that while good correlation between calcification and size existed for both sexes, many individual exceptions were observed.

In this connection if the body weight and sex of 10 birds or more of a single bioassay are known with certainty at four weeks of age, it was found that the ash content may be predicted accurately within fairly narrow limits. The valuable practical application of this principle to the feeding of vitamin-D carriers and to determine subsequently their approximate potency is inferentially obvious.

#### VARIATION IN CALCIFICATION RELATED TO AGE

Previous work from this laboratory (9) (10) has shown that the variation in ash content is similar for both sexes, and the writer has never been able to produce a significantly different result in any of his experiments. This has been amply confirmed by further experimental study, the use of the principle permitting the statistical treatment of bone ash determinations as averages when studying variation, regardless of the bird's sex, and for this reason a greater number of individuals has been made possible for analysis. The variation in bone ash content of more than 2,000 birds at various ages when they ate different forms of vitamin D are presented in Table 4, which includes totals for the various age groups, the several lots being arranged according to age and ash content, the younger chicks first and ranging from the lowest to the highest ash.

When hatched the ash content varied from 33.91 to 34.93 per cent, with the group average 34.45 per cent. The variability (best shown by standard deviation) was unusually low, the similarity indicating that bone ash of chicks newly-hatched is not greatly different.

In considering birds one week old, the average ash content varied from 32.68 to 39.87 per cent, average 37.40 per cent, the variability being somewhat greater than that of those newly hatched. Possibly this increased variation is directly traceable to the changing composition of the bone salts when birds have access to feed, some birds being more efficient in producing well calcified bone than others. In only one pen (Lot 2) was the ash content lower than that of newly hatched chicks, all the other pens showing higher ash, which seems, moreover, dependent upon the form and supply of vitamin D. From this observation it appears clear that the birds received sufficient vitamin (probably in greatest amounts through absorption from the yolk sac or possibly through mobilizable reserves present in the liver or other organic tissues) to prevent the onset of decalcification and corresponding lower ash content. Another possi-



TABLE 4.—*Variation in bone ash content shown by white Leghorns at various ages that received different forms of vitamin D*

| LOT NO.         | SUPPLEMENT ADDED TO A.O.A.C. RATION | AGE          | NO. BIRDS | BONE ASH<br>MEAN | S.D. |
|-----------------|-------------------------------------|--------------|-----------|------------------|------|
| <i>per cent</i> |                                     | <i>weeks</i> |           | <i>per cent</i>  |      |
| 5-14-35         |                                     | 1 day        | 12        | 33.91            | 1.68 |
| 5- 8-35         |                                     | 1 "          | 12        | 34.40            | 1.09 |
| 5-28-35         |                                     | 1 "          | 12        | 34.41            | 1.92 |
| 6-18-35         |                                     | 1 "          | 14        | 34.54            | 1.22 |
| 6-11-35         |                                     | 1 "          | 13        | 34.93            | 1.28 |
| Total           |                                     | 1 "          | 63        | 34.45            | 1.45 |
| 2               | 0.50 low vit. D cod-liver oil       | 1            | 12        | 32.68            | 1.64 |
| 2               | " " " " " "                         | 1            | 11        | 34.35            | 1.62 |
| 1               | 1.00 corn oil                       | 1            | 12        | 35.07            | 2.72 |
| 9               | 0.25 irr. yeast                     | 1            | 12        | 35.77            | 2.24 |
| 7               | 0.28 irr. erg.                      | 1            | 12        | 36.21            | 2.74 |
| 3               | 0.50 sard. (pilchard) oil           | 1            | 11        | 36.23            | 1.89 |
| 4               | 0.13 con. cod-liver oil             | 1            | 11        | 36.40            | 3.01 |
| 3               | 0.50 sard. (pilchard) oil           | 1            | 12        | 36.81            | 1.86 |
| 4               | 0.13 con. cod-liver oil             | 1            | 12        | 36.86            | 1.90 |
| 1               | 1.00 corn oil                       | 1            | 12        | 36.98            | 3.45 |
| 9               | 0.25 irr. yeast                     | 1            | 12        | 37.34            | 2.43 |
| 11              | 0.28 cod-liver oil                  | 1            | 12        | 37.35            | 1.35 |
| 11              | " " "                               | 1            | 12        | 37.36            | 1.38 |
| 5               | 0.50 high vit. D cod-liver oil      | 1            | 12        | 37.64            | 2.25 |
| 12              | 0.28 irr. chol.                     | 1            | 12        | 37.76            | 1.98 |
| 5               | 0.50 high vit. D cod-liver oil      | 1            | 11        | 38.58            | 2.34 |
| 8               | 0.56 irr. erg.                      | 1            | 12        | 38.59            | 2.26 |
| 7               | 0.28 " "                            | 1            | 13        | 38.73            | 2.51 |
| 10              | 0.50 irr. yeast                     | 1            | 12        | 38.90            | 1.78 |
| 6               | 1.00 corn oil, ra. irr.             | 1            | 12        | 39.31            | 1.48 |
| 10              | 0.50 irr. yeast                     | 1            | 12        | 39.42            | 1.67 |
| 12              | 0.28 irr. chol.                     | 1            | 12        | 39.55            | 2.40 |
| 8               | 0.56 irr. erg.                      | 1            | 11        | 39.61            | 1.44 |
| 6               | 1.00 corn oil, ra. irr.             | 1            | 12        | 39.87            | 0.96 |
| T(1-12 inc.)    |                                     | 1            | 284       | 37.40            | 3.70 |
| 2               | 0.50 low vit. D cod-liver oil       | 2            | 11        | 33.52            | 3.21 |
| 1               | 1.00 corn oil                       | 2            | 12        | 34.70            | 4.61 |
| 2               | 0.50 low vit. D cod-liver oil       | 2            | 11        | 35.20            | 3.21 |
| 1               | 1.00 corn oil                       | 2            | 12        | 35.20            | 4.05 |
| 11              | 0.28 cod-liver oil                  | 2            | 12        | 37.27            | 3.79 |
| 3               | 0.50 sard. (pilchard) oil           | 2            | 11        | 38.83            | 1.67 |
| 11              | 0.28 cod-liver oil                  | 2            | 12        | 39.46            | 3.75 |
| 5               | 0.50 high vit. D cod-liver oil      | 2            | 11        | 40.42            | 4.74 |
| 9               | 0.25 irr. yeast                     | 2            | 11        | 40.65            | 4.27 |
| 7               | 0.28 irr. erg.                      | 2            | 13        | 40.74            | 2.04 |
| 7               | " " "                               | 2            | 13        | 41.00            | 3.95 |
| 3               | 0.50 sard. (pilchard) oil           | 2            | 11        | 41.22            | 2.98 |
| 4               | 0.13 con. cod-liver oil             | 2            | 12        | 41.32            | 2.29 |
| 9               | 0.25 irr. yeast                     | 2            | 11        | 41.82            | 1.74 |
| 5               | 0.50 high vit. D cod-liver oil      | 2            | 12        | 41.82            | 2.50 |

TABLE 4.—(Continued)

| LOT NO.      | SUPPLEMENT ADDED TO A.O.A.C. RATION | AGE          | NO.<br>BIRDS | BONE ASH        |      |
|--------------|-------------------------------------|--------------|--------------|-----------------|------|
|              |                                     |              |              | MEAN            | S.D. |
|              | <i>per cent</i>                     | <i>weeks</i> |              | <i>per cent</i> |      |
| 4            | 0.13 con. cod-liver oil             | 2            | 10           | 41.85           | 3.00 |
| 8            | 0.56 irr. erg.                      | 2            | 12           | 42.00           | 3.20 |
| 6            | 1.00 corn oil, ra. irr.             | 2            | 12           | 42.46           | 1.29 |
| 8            | 0.58 irr. erg.                      | 2            | 12           | 42.52           | 1.50 |
| 6            | 1.00 corn oil, ra. irr.             | 2            | 12           | 42.55           | 0.87 |
| 10           | 0.50 irr. yeast                     | 2            | 11           | 42.62           | 1.26 |
| 10           | " " "                               | 2            | 12           | 42.81           | 1.39 |
| 12           | 0.28 irr. chol.                     | 2            | 12           | 43.91           | 2.28 |
| 12           | " " "                               | 2            | 12           | 43.99           | 2.52 |
| T(1-12 inc.) |                                     | 2            | 280          | 40.35           | 4.09 |
| 1            | 1.00 corn oil                       | 3            | 12           | 29.96           | 3.79 |
| 1            | " " "                               | 3            | 11           | 30.43           | 2.87 |
| 2            | 0.50 low vit. D cod-liver oil       | 3            | 11           | 31.83           | 3.34 |
| 2            | " " " " " "                         | 3            | 13           | 31.90           | 2.16 |
| 11           | 0.28 cod-liver oil                  | 3            | 12           | 38.40           | 2.98 |
| 11           | " " "                               | 3            | 12           | 39.35           | 3.23 |
| 7            | 0.28 irr. erg.                      | 3            | 13           | 40.80           | 5.54 |
| 9            | 0.25 irr. yeast                     | 3            | 12           | 41.85           | 3.79 |
| 7            | 0.28 irr. erg.                      | 3            | 12           | 42.39           | 3.49 |
| 3            | 0.50 sard. (pilchard) oil           | 3            | 12           | 43.02           | 3.55 |
| 10           | 0.50 irr. yeast                     | 3            | 12           | 43.08           | 2.99 |
| 10           | " " "                               | 3            | 12           | 43.35           | 1.35 |
| 8            | 0.56 irr. erg.                      | 3            | 12           | 43.37           | 2.23 |
| 3            | 0.50 sard. (pilchard) oil           | 3            | 11           | 43.40           | 2.34 |
| 9            | 0.25 irr. yeast                     | 3            | 12           | 43.49           | 1.59 |
| 5            | 0.50 high vit. D cod-liver oil      | 3            | 11           | 43.79           | 1.71 |
| 4            | 0.13 con. cod-liver oil             | 3            | 11           | 44.19           | 1.44 |
| 8            | 0.56 irr. erg.                      | 3            | 13           | 44.30           | 1.20 |
| 6            | 1.00 corn oil, ra. irr.             | 3            | 12           | 44.30           | 1.16 |
| 6            | " " " " " "                         | 3            | 12           | 44.37           | 1.12 |
| 4            | 0.13 con. cod-liver oil             | 3            | 11           | 44.51           | 1.51 |
| 5            | 0.50 high vit. D cod-liver oil      | 3            | 12           | 44.74           | 0.51 |
| 12           | 0.28 irr. chol.                     | 3            | 12           | 45.68           | 1.34 |
| 12           | " " "                               | 3            | 13           | 45.87           | 0.82 |
| T(1-12 inc.) |                                     | 3            | 286          | 41.21           | 5.46 |
| 1            | 1.00 corn oil                       | 4            | 13           | 30.10           | 1.81 |
| 1            | " " "                               | 4            | 14           | 30.29           | 2.24 |
| 2            | 0.50 low vit. D cod-liver oil       | 4            | 11           | 30.94           | 3.73 |
| 2            | " " " " " "                         | 4            | 12           | 30.94           | 1.30 |
| 11           | 0.28 cod-liver oil                  | 4            | 12           | 38.14           | 3.52 |
| 11           | " " "                               | 4            | 12           | 38.27           | 4.48 |
| 7            | 0.28 irr. erg.                      | 4            | 13           | 40.69           | 2.86 |
| 7            | " " "                               | 4            | 12           | 41.37           | 3.88 |
| 9            | 0.25 irr. yeast                     | 4            | 11           | 43.39           | 1.23 |
| 6            | 1.00 corn oil, ra. irr.             | 4            | 11           | 43.59           | 1.51 |
| 10           | 0.50 irr. yeast                     | 4            | 11           | 43.95           | 2.26 |
| 6            | 1.00 corn oil, ra. irr.             | 4            | 12           | 44.00           | 1.78 |

TABLE 4.—(Continued)

| LOT NO.      | SUPPLEMENT ADDED TO A.O.A.C. RATION             | AGE          | NO.<br>BIRDS | BONE ASH        |      |
|--------------|---|--------------|--------------|-----------------|------|
|              |   |              |              | MEAN            | S.D. |
|              | <i>per cent</i>                                 | <i>weeks</i> |              | <i>per cent</i> |      |
| 9            | 0.25 irr. yeast                                 | 4            | 11           | 44.14           | 2.01 |
| 8            | 0.56 irr. err.                                  | 4            | 11           | 44.20           | 1.50 |
| 8            | " " "   | 4            | 12           | 44.26           | 0.97 |
| 10           | 0.50 irr. yeast                                 | 4            | 11           | 44.46           | 0.86 |
| 3            | 0.50 sard. (pilchard) oil                       | 4            | 11           | 44.76           | 2.33 |
| 3            | " " " "   | 4            | 11           | 44.84           | 2.74 |
| 4            | 0.13 con. cod-liver oil                         | 4            | 11           | 44.90           | 1.32 |
| 4            | " " " "   | 4            | 11           | 45.42           | 1.17 |
| 5            | 0.50 high vit. D cod-liver oil                  | 4            | 10           | 45.86           | 1.56 |
| 12           | 0.28 irr. chol.                                 | 4            | 12           | 46.25           | 0.88 |
| 5            | 0.50 high vit. D cod-liver oil                  | 4            | 12           | 46.28           | 1.19 |
| 12           | 0.28 irr. chol.                                 | 4            | 12           | 46.35           | 0.69 |
| T(1-12 inc.) |   | 4            | 279          | 41.34           | 5.91 |
| c            | none  | 4            | 13           | 27.94           | 1.68 |
| c            | 2.63 charcoal <sup>a</sup>                      | 4            | 13           | 28.76           | 1.00 |
| c            | 1.00 corn oil (ref. cod liver oil)              | 4            | 15           | 29.29           | 2.03 |
| c            | 5.00 irr. yeast (CLO-59)                        | 4            | 11           | 29.56           | 1.29 |
| c            | 10.00 " " " "                                   | 4            | 10           | 29.61           | 2.09 |
| c            | 0.06 con. cod-liver oil (CLO-55)                | 4            | 10           | 29.83           | 2.19 |
| c            | 0.50 cod-liver oil (CLO-103)                    | 4            | 12           | 29.91           | 1.87 |
| c            | 0.06 U.S.P. ref. cod-liver oil                  | 4            | 13           | 30.28           | 2.05 |
| c            | 0.50 cod-liver oil (CLO-105)                    | 4            | 12           | 31.54           | 1.72 |
| c            | 0.13 con. cod-liver oil (CLO-65)                | 4            | 13           | 31.72           | 2.79 |
| c            | 0.38 cod-liver oil +1.13% charcoal <sup>a</sup> | 4            | 28           | 31.87           | 2.24 |
| c            | 1.00 corn oil (ref. cod-liver oil)              | 4            | 16           | 32.20           | 2.22 |
| c            | 0.38 cod-liver oil (CLO-56)                     | 4            | 29           | 32.25           | 1.84 |
| c            | 0.25 " " (CLO-63)                               | 4            | 10           | 33.04           | 3.45 |
| c            | 10.00 cod-liver meal (CLO-88)                   | 4            | 8            | 33.05           | 1.07 |
| c            | 0.25 sard. (pilchard) oil (CLO-95)              | 4            | 11           | 33.20           | 2.59 |
| c            | 0.25 cod-liver oil (CLO-64)                     | 4            | 10           | 33.47           | 2.96 |
| c            | 0.13 con. cod-liver oil (CLO-72)                | 4            | 12           | 33.48           | 2.46 |
| c            | 0.06 " " " (CLO-109)                            | 4            | 11           | 33.74           | 1.78 |
| c            | 0.50 cod-liver oil (CLO-106)                    | 4            | 13           | 33.89           | 2.35 |
| c            | 1.00 corn oil                                   | 4            | 19           | 34.09           | 1.94 |
| c            | 0.50 cod-liver oil (CLO-96)                     | 4            | 11           | 34.10           | 5.05 |
| c            | 0.13 U.S.P. ref. cod-liver oil                  | 4            | 13           | 34.17           | 2.12 |
| c            | 5.00 cod-liver meal (CLO-88)                    | 4            | 10           | 34.33           | 2.31 |
| c            | 0.50 cod-liver oil (CLO-90)                     | 4            | 12           | 34.82           | 3.86 |
| c            | 0.13 con. cod-liver oil (CLO-55)                | 4            | 13           | 36.48           | 4.44 |
| c            | 0.63 cod-liver oil +1.88% charcoal <sup>a</sup> | 4            | 26           | 36.61           | 3.41 |
| c            | 0.25 cod-liver oil (CLO-108)                    | 4            | 12           | 36.79           | 3.33 |
| c            | 0.50 " " (CLO-74)                               | 4            | 11           | 37.02           | 3.49 |
| c            | 0.63 " " (CLO-56)                               | 4            | 27           | 37.06           | 3.80 |
| c            | 0.13 con. cod-liver oil (CLO-67)                | 4            | 12           | 37.51           | 4.36 |
| c            | 0.25 sard. (pilchard) oil (CLO-61)              | 4            | 12           | 37.61           | 3.05 |
| c            | 0.50 cod-liver oil (CLO-63)                     | 4            | 11           | 37.79           | 4.90 |
| c            | 0.50 sard. (pilchard) oil (CLO-57)              | 4            | 12           | 37.96           | 3.66 |

TABLE 4.—(Continued)

| LOT NO. | SUPPLEMENT ADDED TO A.O.A.C. RATION              | AGE          | NO.<br>BIRDS | BONE ASH        |      |
|---------|--|--------------|--------------|-----------------|------|
|         |  |              |              | MEAN            | S.D. |
|         | <i>per cent</i>                                  | <i>weeks</i> |              | <i>per cent</i> |      |
| c       | 0.25 " " (CLO-91)                                | 4            | 10           | 38.35           | 3.24 |
| c       | 0.50 cod-liver oil (CLO-56)                      | 4            | 10           | 38.42           | 3.60 |
| c       | 1.00 " " (CLO-90)                                | 4            | 13           | 38.91           | 4.26 |
| c       | 0.06 con. cod-liver oil (CLO-71)                 | 4            | 10           | 40.80           | 5.30 |
| c       | 0.13 " " (CLO-87)                                | 4            | 10           | 40.86           | 4.08 |
| c       | 0.50 cod-liver oil (CLO-64)                      | 4            | 12           | 41.50           | 3.74 |
| c       | 0.50 " " (CLO-104)                               | 4            | 12           | 41.53           | 3.71 |
| c       | 0.50 sard. (pilchard) oil (CLO-100)              | 4            | 11           | 41.79           | 4.90 |
| c       | 0.25 U.S.P. ref. cod-liver oil                   | 4            | 13           | 42.55           | 4.04 |
| c       | 0.88 cod-liver oil (CLO-56)                      | 4            | 26           | 42.64           | 3.64 |
| c       | 0.19 U.S.P. ref. cod-liver oil                   | 4            | 13           | 43.03           | 3.25 |
| c       | 0.19 con. cod-liver oil (CLO-84)                 | 4            | 11           | 43.17           | 2.59 |
| c       | 0.88 cod-liver oil + 2.63% charcoal <sup>a</sup> | 4            | 27           | 43.17           | 3.06 |
| c       | 0.50 " " (CLO-97)                                | 4            | 11           | 44.40           | 2.97 |
| c       | 1.00 " " (CLO-96)                                | 4            | 13           | 44.60           | 2.93 |
| c       | 0.50 " " (CLO-75)                                | 4            | 12           | 45.10           | 1.96 |
| c       | 0.06 con. cod-liver oil (CLO-89)                 | 4            | 9            | 45.49           | 3.06 |
| c       | 0.25 cod-liver oil (CLO-85)                      | 4            | 13           | 45.51           | 1.15 |
| c       | 0.06 con. cod-liver oil (CLO-51)                 | 4            | 12           | 45.62           | 1.91 |
| c       | 0.50 cod-liver oil (CLO-76)                      | 4            | 12           | 45.67           | 1.16 |
| c       | 0.25 U.S.P. ref. cod-liver oil                   | 4            | 12           | 45.71           | 2.23 |
| c       | 0.25 con. cod-liver oil (CLO-55)                 | 4            | 8            | 45.86           | 1.89 |
| c       | 0.50 cod-liver oil (CLO-82)                      | 4            | 12           | 46.08           | 0.91 |
| c       | 0.50 sard. (pilchard) oil (CLO-61)               | 4            | 13           | 46.08           | 3.36 |
| c       | 1.00 cod-liver oil (CLO-56)                      | 4            | 11           | 46.19           | 2.15 |
| c       | 0.13 con. cod-liver oil (CLO-51)                 | 4            | 12           | 46.42           | 1.15 |
| c       | 0.29 U.S.P. ref. cod-liver oil                   | 4            | 13           | 46.46           | 2.29 |
| c       | 1.00 cod-liver oil (CLO-74)                      | 4            | 10           | 46.52           | 0.86 |
| c       | 0.25 composite of 13<br>cod-liver oils (CLO-54)  | 4            | 12           | 46.57           | 0.74 |
| c       | 0.38 U.S.P. ref. cod-liver oil                   | 4            | 13           | 46.60           | 1.03 |
| c       | 0.50 " " " "                                     | 4            | 13           | 46.81           | 1.37 |
| c       | 0.38 " " " "                                     | 4            | 13           | 46.99           | 1.11 |
| c       | 0.25 cod-liver oil (CLO-78)                      | 4            | 11           | 47.00           | 2.65 |
| c       | 0.33 U.S.P. ref. cod-liver oil                   | 4            | 13           | 47.03           | 0.94 |
| c       | 0.50 sard. (pilchard) oil (CLO-60)               | 4            | 10           | 47.11           | 2.53 |
| c       | 0.50 cod liver oil (CLO-77)                      | 4            | 11           | 47.13           | 1.23 |
| c       | 0.25 " " (CLO-69)                                | 4            | 12           | 47.18           | 0.91 |
| c       | 0.50 " " (CLO-73)                                | 4            | 13           | 47.19           | 1.07 |
| c       | 1.00 sard. (pilchard) oil (CLO-57)               | 4            | 10           | 47.23           | 3.08 |
| c       | 0.50 cod-liver oil (CLO-68)                      | 4            | 13           | 47.33           | 0.90 |
| c       | 1.00 composite of 13<br>cod-liver oils (CLO-54)  | 4            | 12           | 47.46           | 1.08 |
| c       | 0.25 con. cod-liver oil (CLO-51)                 | 4            | 7            | 47.61           | 0.77 |
| c       | 3.00 prop. prod. (CLO-58)                        | 4            | 11           | 47.78           | 0.52 |
| c       | 0.50 composite of 13<br>cod-liver oils (CLO-54)  | 4            | 12           | 47.79           | 0.60 |

<sup>a</sup> Substituted for an equivalent amount of corn meal.

bility that may also be considered is that birds of this tender age (one week), eating feed supplied the first week, in all probability obtain a small amount of vitamin from the feed consumed, and that this is well utilized for the production of calcified bone. When variability (standard deviation) of ash content is observed for birds in these lots, a noticeable decrease was not evident throughout, the various lots exhibiting considerable lack of uniformity in this respect.

Considering birds two weeks old, it will be noted that very little loss in ash content is apparent even when the diet was quite low in the supply of vitamin, while lots fed adequate amounts, *i.e.*, Nos. 12 and 5, produced an increased calcification, undoubtedly due to the extra vitamin consumed during this time. Variability in ash content showed an increase over that of birds one week old and decreased somewhat when they were fed increased amounts of vitamin, although many exceptions to this observation will be noted. That a real increase in variability occurred is further evident when the totals for the one- and two-week groups are compared.

The variability in ash content for birds three weeks old was considerably higher than that previously shown by the other age groups, ranging from 0.51 to 5.54, average 5.46, while the ash content averaged 29.96 to 45.87 per cent, showing that variability apparently decreased when ash content was normal or nearly so. The pens given corn oil and low vitamin-D cod-liver oil (Lots 1 and 2) have significantly lower ash content than birds newly hatched, no doubt showing the effects of vitamin deprivation. It may be concluded from this evidence that vitamin deficiency is not apparent by calcification produced until at least three weeks, prior to this time the supply other than the small amount present in the feed adequately maintaining a bone ash equal to that possessed by birds when hatched. The limits to which bone ash may be reduced, unless other outside sources of vitamin are removed, presumably through post-operative removal of the yolk sac or other stored vitamin sources, are placed under these conditions at three weeks' duration. It is evident also that bone ash content may be increased over that of newly hatched birds prior to this time. The differences in ash content between birds at different ages supplied adequate vitamin and those deprived of this factor were as follows: one week, 7.19 per cent; two weeks, 10.47 per cent; three weeks, 15.91 per cent; and four weeks, 16.25 per cent. It is clear that the difference increased rapidly to three weeks and very slowly thereafter.

The same general trends of bone ash variability are shown at four weeks as at three weeks, varying 0.69 to 4.48, average of the group 5.91, the latter figure being slightly higher than at three weeks. Bone ash content varied from 30.10 to 46.35 per cent, average 41.34 per cent.

Variations in several routine bioassays (Lot c) are presented graphically in Fig. 2 for comparison with the 12 experimental lots, the ash ranging

from 27.94 to 47.79 per cent. It seems apparent that pens in the same lot may show similar ash content but different variability. Histograms made for Lot c reveal the striking fact that variability remained low when calcification was deficient at least up to and including 35 per cent bone ash, and increased gradually and uniformly to a maximum of 40 per cent and thereafter gradually receded to lower levels when bone ash content reached 45 per cent or more. It seems clear that variability was greatest when bone ash content was subnormal and lowest at percentages greater than 45 and less than 35, this observation being in accord with the results of several other investigations of a like nature that were conducted previously (14). The curves support the supposition that variation in ash content decreases not only with increasing but also with *decreasing*, amounts of vitamin, and reaches a peak at approximately 40 per cent, and that these progressions in bone ash variation are more or less uniform in character.

#### SOURCE OF ERRORS

The data presented seem to furnish strong support for several suppositions, *e.g.*, (1) The size (body weight) criterion has an important supplementary value, but its degree of accuracy is less than that of the calcification (bone ash determinations) criterion; (2) the variation in response of chicks at various ages to biological test is the chief source of error, effect of sex upon results being practically negligible; (3) provided all substances necessary for growth other than vitamin D are abundantly supplied, increase in chick body weight is directly related to the dose of vitamin given;<sup>1</sup> (4) size (body weight) and calcification (bone ash content) variations are similar for the sexes at different ages of the chick; (5) reducing the feeding period from four to three weeks does not cause an appreciably greater error in the results of bioassay at the shorter feeding interval; (6) greater variability and consequently more opportunity for error exists when the average bone ash content of a bioassay group is between 35 per cent and 45 per cent than below 35 per cent or greater than 45 per cent.

#### SUMMARY AND CONCLUSIONS

The effect of different ages (1 day to four weeks), sex, size (body weight), and calcification (bone ash content) of young chicks on accuracy of A.O.A.C. preventive bioassay has been critically studied by statistically examining data from more than two thousand birds fed various vitamin-D supplements. The results obtained under the conditions of the experiment reported are as follows:

1. Absence of vitamin D rather than age caused the normal sex difference in calcification favoring females to reverse to one favoring males.

<sup>1</sup> In agreement with the results found by Coward, Key, and Morgan (15) with rats that had ceased growing through vitamin depletion and had subsequently received daily doses of vitamin, the rate increasing in weight in proportion to the amount of vitamin received.

2. Correlations between size and calcification were positively significant for both sexes at various ages, but showed no consistent sex differences, heavier birds producing a higher ash content than lighter ones.

3. The size criterion for interpreting results of bioassay has an important supplementary value, but the degree of accuracy was less than when the calcification criterion was used.

4. Calcification did not decrease as the result of vitamin D deficiency until the birds were three weeks old, but it may increase prior to this time if the birds receive a sufficient supply of vitamin.

5. Differences in calcification occurred at all ages (except those newly hatched) and were most pronounced at three and four weeks, after three weeks very little apparent increase in calcification differences occurring.

6. Variation for both size and calcification was similar for the sexes at different ages.

7. The chief source of errors in bioassay was the variation in response of chicks at various ages to biological means of testing, the effect of sex in birds aged four weeks or younger upon results being negligible if average ash content was employed.

8. Judged from calcification criterion the error of bioassay was inversely proportional to the square of the number of chicks employed, trustworthy results being obtained with 10 chicks of either one or both sexes per bioassay.

9. In reducing the length of test period one week, the error involved was no greater than that at four weeks.

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## BOOK REVIEWS

**Introduction to Theoretical Chemistry.** By WILLIAM BUELL MILDRED, Professor of Chemistry at Haverford College, and FRANK THOMSON GUCKER, JR., Associate Professor of Chemistry at Northwestern University. XIV+614 pp. American Book Co., New York. 1936. Price \$3.50.

This review of *Introduction to Theoretical Chemistry* may well be introduced by quoting from the preface: "General chemistry has grown to be too big a subject. During the past decade the number of topics to be studied has greatly increased. Many topics from the field of physical chemistry formerly considered distinctly 'advanced' have crowded in and new developments have been added. Theory content as well as fact content has grown. \* \* \* The authors have applied themselves to outline a course of study which will amplify the too thinly disseminated knowledge of important generalizations already possessed by the student and to bring into a unified whole the basic principles of chemistry and their relationships."

The authors have accomplished what they intended, and have succeeded in producing a textbook for beginners in theoretical chemistry that should find ready acceptance.

The elements and their classification are discussed in the first chapter, after which follow 22 chapters dealing with practically every subject ordinarily included in a course in general and elementary physical chemistry. The chapter on fundamental chemical theory is particularly well done. Three chapters, or over 100 pages, are devoted to the theory and application of ionization, and four chapters (104 pages) to electrochemistry. These are followed by a chapter on modern theories of electrolytes, in which is included a criticism of Arrhenius' theory of ionization. One wonders whether it would not have been advisable to criticize Arrhenius' theory when it was first presented and discussed (chap. 8). Such topics as radiation and spectra, radioactivity, isotopes, atomic structure, and the electronic theory of valence are considered in the last 160 pages. All these chapters are clear and well presented. The reviewer feels, however, that more space should have been allotted to the discussion of X-rays. The calculations necessary for determining distances between atomic planes, as developed by Bragg, would be of considerable interest and value to the student, and should have been included. A chapter on elementary thermodynamics would also have contributed to the completeness of the book.

The style is clear and the subject matter is readily understood. Many of the chapters are introduced by a brief historical discussion—a commendable feature—and all of them end with a summary and a list of references for outside reading.

The reviewer considers this a splendid textbook, and does not hesitate to recommend it to teachers of elementary theoretical chemistry.—O. A. NELSON.

**The Bacteriological Grading of Milk.** By G. S. WILSON, M.D. Medical Research Council, 1935. Special Report Series No. 206. London. Price 7s. 6d. net.

This report, presenting the results of an investigation conducted by Professor Wilson and several assistants over a period of three and one-half years, gives critical consideration to the value of the bacteriological methods commonly employed for assessing the cleanliness and keeping quality of milk. At the outset the author makes it plain that the safety of milk, as shown by the absence of pathogenic organisms, is not to be discussed. The conclusions reached strongly condemn the use of the plate count test in the bacteriological grading of milk. Discontinuance of this method for such a purpose is recommended. The sediment test, the leucocyte count, the titratable acidity, the H-ion concentration, the increase in acidity, the brom-



thymol blue test and the keeping quality test are likewise considered unsuitable for the routine grading of milk. Although considerable merit is ascribed to the direct microscopic, or Breed smear, method, particularly in view of the rapidity with which results differentiating between clean and dirty milks can be obtained, the method is held to be less suitable for grading purposes than a modified methylene blue reduction test described in the report. It is maintained that the modified methylene blue reduction method is a simple and inexpensive test with a very small experimental error and can be carried out by relatively unskilled workers on large numbers of samples with a minimum of equipment.

The report emphasizes the weaknesses in present established procedures and evaluates, in great detail, the numerous factors contributing to the shortcoming of these methods. Those interested in the control of production and distribution of milk may find in this painstaking and elaborate investigation a distinct challenge to defend certain present-day methods generally accepted as standard procedures. Because of the nicety of the experimental work, the clarity of presentation, and the thought-stimulating character of the comments and conclusions, this book of 392 pages is a valuable contribution to the literature on dairy bacteriology.—A. C. HUNTER.

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